Monthly Progress Report

RECD
3/8/91
F.B.

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I.D. NO.: RIDO01194323
FILE NO.: R-9
OTHER:

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Pursuant to: RCRA I-88-1088

Facility Site: Cranston, RI

Period Covered: February 1991 (26 January 1991 – 22 February 1991)*

Date Submitted: 10 March 1991

1.0 SUMMARY

This is the eighth monthly progress report. Seven significant events occurred this month. These events are summarized in this section and discussed in detail in later sections of this report.

Round 1 Analyses (Task 5.7). Reduction and interpretation of field data continued; analyses of soil, sediment, and water samples from Round 1 continued. Unvalidated Round 1 analytical data were reviewed in developing the proposed Round 2 river sampling strategy (Attachment A). A report describing the bioassay results from Round 1 samples was completed (Attachment B).

Quality Assurance (Task 12). Data validation was completed for selected samples and continued for other samples. Developing the database for storing and analyzing project data continued; loading the project database continued. Additional revisions to the Region I data validation worksheets were requested by USEPA. The worksheets were revised and submitted to USEPA on schedule as part of the revised RFI Proposal.

Water Level Monitoring. Monthly groundwater level monitoring continued.

Pump Tests. Reduction of the mini-pump test data continued.

RFI Proposal. The revised RFI Proposal was submitted to USEPA on schedule (2/8/91).
Change in Plan: The USEPA requested a proposal for sampling the Pawtuxet River; the proposed Round 2 river sampling strategy is presented in Attachment A. [Section 8.0 discusses changes in the Work Plan.]

Round 2 Sampling. Mobilization (e.g., preparing bid packages, negotiating contracts with subcontractors, updating Health & Safety Plans) for Round 2 sampling began.

*As agreed, the reporting period will be monthly through the fourth Friday of the month.

Interim Report. The preliminary outline was revised; drafting selected sections of the Interim Report continued.

2.0 TASKS AND ACTIVITIES COMPLETED

The sampling and other activities (subtasks) that were completed are reported here.

2.1 Sampling Activities Completed

No sampling activities were conducted in this reporting period.

2.2 Other Activities Completed

Other activities (subtasks) were completed within several tasks.

Round 1 Analyses (Task 5.7). Unvalidated Round 1 analytical data were reviewed in developing the proposed Round 2 river sampling strategy presented in Attachment A. The bioassay results from Round 1 samples were completed; a report describing those results is included as Attachment B.

Quality Assurance (Task 12). Data validation for selected samples was completed. Additional revisions to the Region I data validation worksheets were requested by USEPA; the due date for submitting the worksheets was extended to 2/8/91 to accommodate these revisions. The worksheets were revised and submitted to USEPA on schedule as part of the revised RFI Proposal.

RFI Proposal. The revised RFI Proposal was submitted to USEPA on schedule (2/8/91).

Interim Report. The preliminary outline for the Interim Report was revised.

3.0 JEOPARDY TASKS (scheduled tasks not completed)

No tasks were in jeopardy as of 22 February 1991.

4.0 OTHER TASKS UNDERWAY (and on schedule)

The tasks that were underway (and on schedule as of 22 February 1991) are reported here.

Round 1 Analyses (Task 5.7). Reduction and interpretation of field data continued; analyses of soil, sediment, and water samples from Round 1 continued.

Quality Assurance (Task 12). Data validation continued. Developing the database for storing and analyzing project data continued; loading Round 1 data continued.

Water Level Monitoring. Monthly groundwater level monitoring continued.

Pump Tests. Reduction of the mini-pump test data continued.

Round 2 Sampling. Mobilization (e.g., preparing bid packages, negotiating contracts with subcontractors, updating Health & Safety Plans) for Round 2 sampling began.

Interim Report. Drafting selected sections of the Interim Report continued.

5.0 DATA OBTAINED

The sampling results and other data obtained are reported here.

5.1 Sampling Results

Analytical laboratory data have been received; selected samples have been validated. Bioassay data are presented in Attachment B. Summaries of validated Round 1 data will be included in future Monthly Progress Reports.

5.2 Other Data Obtained

No field logs or other data were obtained during this reporting period.

6.0 PROBLEM AREAS

The resolved, new, and potential (i.e., anticipated or possible) problem areas are reported here.

6.1 Resolved Problem Areas

There were no existing problem areas to resolve during this reporting period.

6.2 New Problem Areas

No new problem areas were identified during this reporting period.

6.3 Potential Problem Areas

Four potential problem areas were identified during this reporting period.

Development of the Project Database Delayed

Development of the project database is proceeding more slowly than anticipated; continued delay in loading the database could delay completing other tasks.

Review of the Problem. The Round 1 analytical laboratory data were received in a format incompatible with that required by the database software, so the database software had to be modified. As a result, loading and analyzing the laboratory data has been delayed, and the Round 1 analytical data have not yet been evaluated. Since Round 2 activities depend on evaluating the Round 1 data, this delay in loading the database and evaluating the Round 1 data could impact the Round 2 task schedule.

Plans for Resolution. The software modifications necessary to resolve the format incompatibilities are underway. After the modified database software is operational and the Round 1 data have been loaded, those data will be evaluated.

Geotechnical Analyses of Round 1 Soil and Sediment Samples Delayed

Only the saturated soil samples have been submitted for geotechnical analysis.

> ***Review of the Problem.*** All saturated soil samples (collected below the water table) have been submitted for geotechnical analysis. Both the unsaturated soil samples (collected above the water table) and the sediment samples cannot be submitted for geotechnical analysis until 1) the analytical results from the Round 1 samples have been evaluated, 2) the nature of the contamination has been determined, and 3) the appropriate laboratory procedures for handling those contaminants have been implemented. Evaluating the Round 1 analytical results depends on the project database being loaded. Since loading the database has proceeded more slowly than expected, geotechnical analysis of Round 1 samples may be delayed.
>
> ***Plans for Resolution.*** The Round 1 data will be loaded and evaluated as soon as possible. Then the Round 1 contaminants will be identified, the appropriate procedures for handling those contaminants will be determined and implemented, and the unsaturated soil samples and sediment samples will be submitted for geotechnical analysis.

Starting Round 2 River Sampling Delayed

Starting Round 2 river sampling has been postponed.

> ***Review of the Problem.*** Because Round 2 sediment and surface water sampling of the Pawtuxet River depends on USEPA approval of the proposed river sampling strategy (Attachment A), starting Round 2 river sampling has been postponed.
>
> ***Plans for Resolution.*** Round 2 sampling will begin after the USEPA approves the proposed river sampling strategy.

Starting Round 2 Soil Sampling Delayed

Because Round 2 sampling depends on analysis and evaluation of the Round 1 data, which in turn depend on loading the project database, starting Round 2 soil sampling has been delayed.

> ***Review of the Problem.*** Round 2 sampling is designed to confirm the results from Round 1 and to characterize the extent of contamination, so the Round 2 sampling strategy depends in part on the results from Round 1. The Round 2 sampling strategy cannot be developed fully until the Round 1 data have been evaluated. The Round 1 data are being loaded into the project database, but have not been evaluated. Consequently, starting Round 2 on-site soil sampling has been postponed from 3/4/91 to 3/11/91; starting Round 2 off-site soil sampling has been postponed from 3/18/91 to 3/25/91.
>
> ***Plans for Resolution.*** After the Round 1 data have been loaded and evaluated, the locations for Round 2 on-site samples and the list of analytes for Round 2 off-site samples will be specified. Then Round 2 soil sampling will begin.

7.0 PROJECTED SCHEDULE OF TASKS (next two months)

The projected schedule (based on Figure 5-2 in Volume 1, Chapter 2 of the *RCRA Facility Investigation Proposal*) is provided here. It covers the tasks to be performed in the next two months (March and April 1991), along with other comments or considerations.

Target Date	Task#	Task	Comments/Considerations
2/27/91	5.7	Round 1 Analyses	
3/11/91	5.8	Round 1 Data Validation	
3/25/91	5.3	Round 2 Sediment Sampling	
3/11/91	5.11	Round 2 Soil Sampling (On-Site)	
3/25/91	5.11	Round 2 Soil Sampling (Off-Site)	
4/10/91	8	March Progress Report	
4/15/91	5.12	Round 2 Groundwater Sampling	
ongoing	9	Project Management	
ongoing	10	Data Management	
ongoing	11	Project Administration	
ongoing	12	Quality Assurance	
ongoing	13	Health & Safety Assurance	

8.0 CHANGES IN WORK PLAN

Changes to Phase IB of the Work Plan based on the findings from Phase IA were recommended in the Phase IA Report (submitted 10/24/90). One additional change to Phase IB of the Work Plan was made during this reporting period and is described here.

RFI Proposal. The USEPA requested a proposal for sampling the Pawtuxet River; the proposed Round 2 river sampling strategy is presented in Attachment A. The strategy is based on a preliminary review of unvalidated analytical data from Round 1 river sediment and surface water sampling. Round 2 river sampling will begin after the USEPA approves the river sampling strategy.

9.0 OTHER COMMENTS

The plans going forward into March and April include:

- continuing the Phase IB field investigation,
- reducing and validating the data collected, and
- continuing to develop the Interim Report.

The following documents are appended:

- Attachment A — Proposal for Sampling of the Pawtuxet River: Phase IB, Round 2, and
- Attachment B — Bioassay of the Toxicity of Sediments, Pore Waters, and Surface Waters Collected from the Pawtuxet River Near the Former Facility of CIBA-GEIGY Corporation at Cranston, Rhode Island).

ATTACHMENT A

SAMPLING STRATEGY FOR THE PAWTUXET RIVER: PHASE IB, ROUND 2

RCRA FACILITY INVESTIGATION CIBA-GEIGY FACILITY CRANSTON, RHODE ISLAND

SUMMARY

This document describes the sampling strategy for the Pawtuxet River in Round 2 of Phase IB of the RCRA Facility Investigation. Unvalidated Round 1 analytical data were reviewed in developing this Round 2 sampling strategy.

The river sampling strategy incorporates four elements:

- o confirmation of selected Round 1 results;
- o delineation of contamination near the Production Area bulkhead;
- o additional sampling locations between the facility reach and the furthest downstream Round 1 sample (SD-20M); and
- o additional bioassay testing.

Surface water samples will be collected from five locations in the river and submitted for the full analyte list (i.e., Appendix IX compounds, fingerprint compounds, physicochemical parameters, major/minor ions, geotechnical parameters, nutrients, and total suspended solids).

Riverbed sediment samples will be collected from fourteen locations in the river and submitted for the full analyte list (i.e., the same as for surface water samples except total suspended solids). In addition, each of these sediment samples will be submitted for the 10 day acute toxicity bioassay using chironomid (midge) larvae.

Finally, 28 sediment samples will be collected from the river near the Production Area bulkhead and analyzed for total petroleum hydrocarbons

(TPHs) and total polynuclear aromatic hydrocarbons (TPAHs).

PRELIMINARY REVIEW OF ROUND 1 ANALYTICAL RESULTS

Figures 1 and 2 show the river locations and media (i.e., surface water and sediment) sampled for Round 1 (26-30 November 1990).

Surface Water

Only trace quantities (i.e., a few ppb) of the following volatile organic compounds were detected in Round 1 surface water samples: toluene, chlorobenzene, xylenes, and methylene chloride. There is no spatial trend apparent in these data. No other Appendix IX contaminants were detected in the Round 1 surface water samples.

Sediment

Contaminants were detected in the far upstream sediment sample (SD-00M) but at concentrations lower than at other locations. Volatile organic and fingerprint compounds were not present in sample SD-00M, but various base neutral organic compounds were detected in concentrations less than a ppm.

An additional upstream sediment sample was collected between the Bellefont Pond inlet and the CIBA-GEIGY facility (SD-01R). Contaminants detected in this sample include volatile organic compounds (in concentrations less than a ppm) and base neutral organic compounds (in concentrations less than 10 ppm).

Four samples were collected near the Production Area bulkhead (SD-02R, SD-02L, SD-03R, and SD-03L). As shown in Figure 2, two of the sample locations are immediately adjacent to the bulkhead (SD-02R and SD-03R); the other two sample locations are across the river from the bulkhead (SD-02L and SD-03L). The samples collected adjacent to the bulkhead contained volatile and base neutral organic compounds in concentrations ranging from less than a ppm to hundreds of ppm. Polychlorinated biphenyls (PCBs) were detected, in concentrations ranging from less than 10 ppm to hundreds of ppm. Metal contaminants detected included arsenic, beryllium, cadmium, chromium, copper, lead, nickel, silver, and zinc. A few ppb of dioxins/furans were present in SD-03R. Tinuvin 327 (a fingerprint compound) was present in SD-03R in

hundreds of ppm.

The sediment samples collected across the river from the bulkhead contained fewer Appendix IX compounds and lower concentrations of contaminants than those adjacent to the bulkhead. Concentrations of volatile organic compounds were less than one-tenth ppm. Concentrations of base neutral compounds ranged from less than one to less than 10 ppm. No PCBs or dioxin/furan compounds were detected. Concentrations of arsenic, beryllium, cadmium, chromium, copper, and zinc were relatively high in SD-02L, but only small quantities of metals were detected in SD-03L.

Generally, the samples downstream from the Production Area bulkhead showed lower concentrations of contaminants. Various base neutral organic compounds were detected in SD-06R, SD-07L, and SD-10M in concentrations less than 10 ppm. Pesticide concentrations in SD-05L, SD-06R, SD-08M, SD-10M, and SD-20M ranged up to tens of ppb. Fingerprint compounds were detected in SD-05L, SD-07L, SD-10M, and SD-20M. The only exceptions to the trend of decreasing contaminant levels downstream from the Production Area were the dioxin/furan results--dioxin/furan compounds were detected at SD-05L and SD-07L in concentrations up to a few ppm.

SAMPLING STRATEGY FOR ROUND 2 -- SURFACE WATER

A preliminary review of the unvalidated Round 1 analytical results indicates only minor contamination of the Pawtuxet River surface water. Therefore, surface water will be collected in Round 2 at five locations on the Pawtuxet River (Figures 3 and 4). These locations were selected to provide coverage in:

- the far upstream area (SW-00MA2);
- the upstream area between Bellefont Pond and the CIBA-GEIGY facility (SW-01M2);
- the facility reach (SW-04M2 and SW-09M2); and
- the area downstream of the facility (SW-20M2).

Two of the Round 2 surface water sample locations were sampled in Round 1 (SW-01M2 and SW-20M2). When compared to Round 1 data, the Round 2 analytical results from these two locations will suggest a range of variability to be expected in the surface water chemistry over time.

SAMPLING STRATEGY FOR ROUND 2 -- SEDIMENTS

A preliminary review of the unvalidated Round 1 analytical results from sediment samples indicated the need for confirmation sampling, delineation sampling, and additional sampling locations. Additional bioassay testing also will be conducted.

Confirmation Sampling

Eight Round 2 sediment samples will provide confirmation samples: SD-01R2, SD-02R2, SD-02L2, SD-03R2, SD-03L2, SD-07L2, SD-08M2, and SD-20M2. Analytical results from these Round 2 samples either will confirm the presence and concentrations of the contaminants observed in Round 1, or will suggest the minimum small-scale variability in the river sediment chemistry. All eight confirmation sediment samples will be analyzed for the full list (i.e., Appendix IX compounds, fingerprint compounds, physicochemical parameters, major/minor ions, geotechnical parameters, and nutrients.)

Additional Sampling Locations

Two new downstream sampling locations (SD-13M2 and SD-16M2) will be used to investigate conditions between the facility reach and the furthest downstream location sampled in Round 1. Results from samples collected at these locations will fill a data gap.

Four new sampling locations along the facility reach (SD-04M2, SD-04R2, SD-05M2, and SD-09R2) will also fill data gaps. These locations were not previously sampled due to difficult sampling conditions.

All six sediment samples from additional sampling locations will be analyzed for the full analyte list (i.e., Appendix IX compounds, fingerprint compounds, physicochemical parameters, major/minor ions, geotechnical parameters, and nutrients.)

Delineation Sampling

Both the relatively high contaminant levels near the Production Area bulkhead and the significantly lower contaminant levels across the river warrant further investigation. A delineation sampling program will investigate the areal extent and continuity of contaminated sediments near the bulkhead.

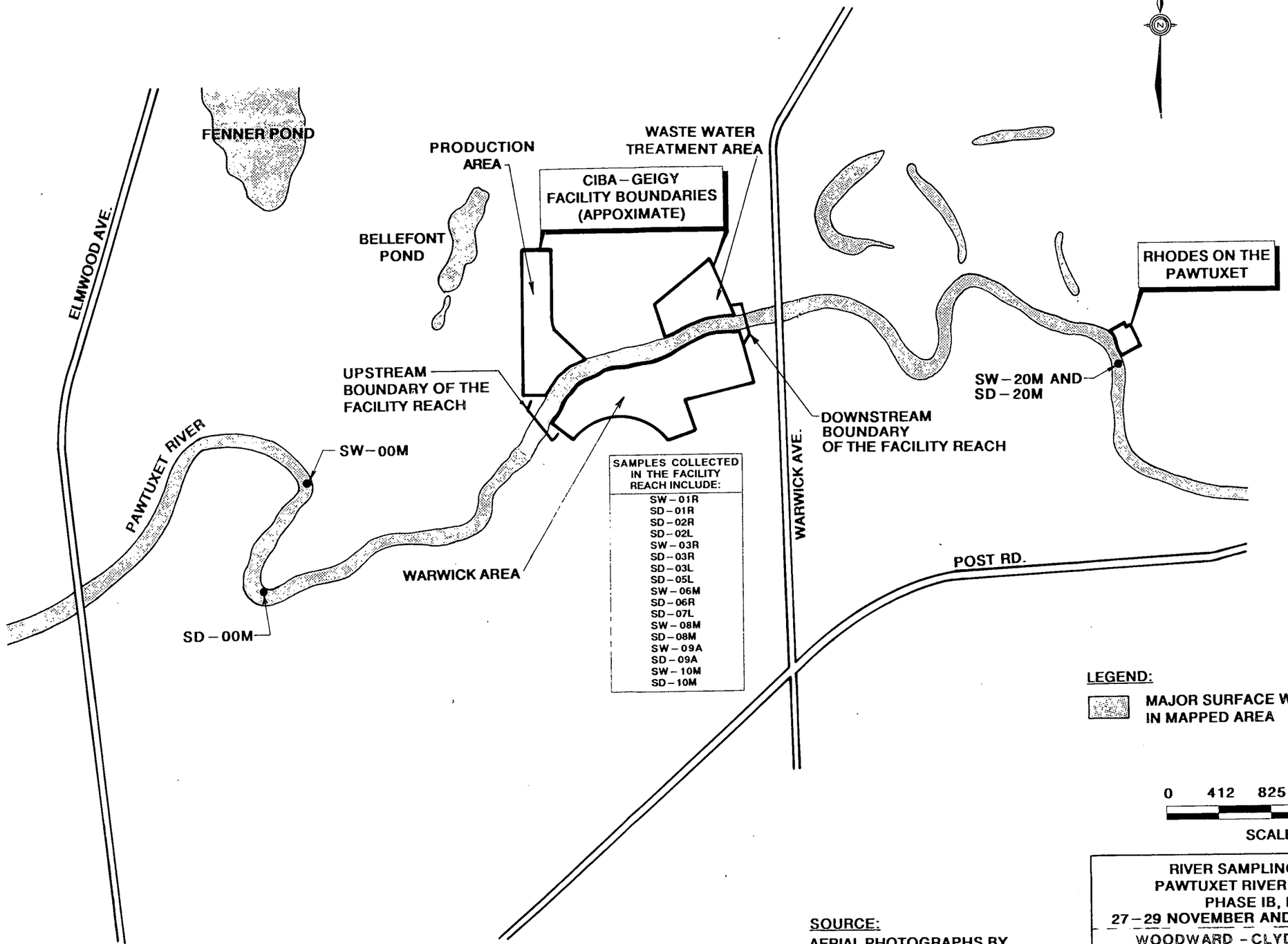
Sampling will be conducted along seven transects shown in Figure 5; each transect will have four evenly spaced sampling locations. Two of these transects were sampled in Round 1 (TE-02 and TE-03). Three new transects have been designated between TE-02 and TE-03 (TE-02A, B, and C). In addition, one transect has been added upstream (TE-01A) and another downstream (TE-03A) of the transects sampled in Round 1. The 28 delineation sediment samples will be analyzed for TPHs and TPAHs.

Bioassay testing

All Round 2 sediment samples (except delineation samples) will be submitted for the 10 day acute toxicity bioassay using chironomid (midge) larvae (see Attachment B to this February Monthly Progress Report).

SCHEDULE

Subcontractors are scheduled to begin this work on 25 March 1991; the field work should be completed in two to three weeks.



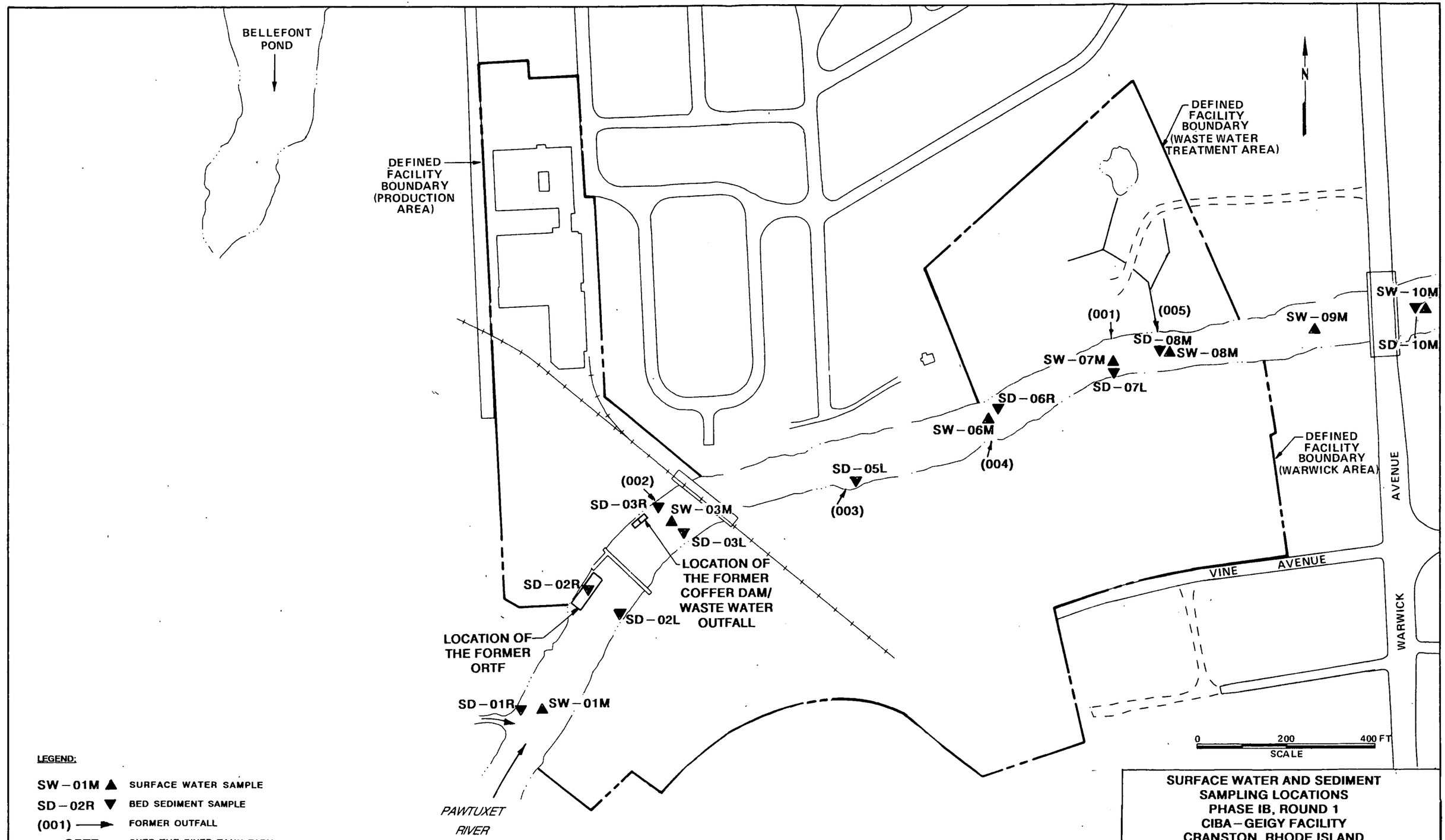
SOURCE:
AERIAL PHOTOGRAPHS BY GEOD CORPORATION OF NEWFOUNDLAND, NEW JERSEY.
DATE FLOWN: 2 APRIL 1989

RIVER SAMPLING LOCATIONS
PAWTUXET RIVER, CRANSTON, RI
PHASE IB, ROUND 1
27 – 29 NOVEMBER AND 7 DECEMBER, 1990

WOODWARD – CLYDE CONSULTANTS

CONSULTING ENGINEERS, GEOLOGISTS AND ENVIRONMENTAL SCIENTISTS
WAYNE, NEW JERSEY

DR.	MG	SCALE 1" ≅ 825 FEET	87X4660
APP.	MEPD	DATE 12 FEB 1991	1



LEGEND:

SW – 01M ▲	SURFACE WATER SAMPLE
SD – 02R ▼	BED SEDIMENT SAMPLE
(001) ⟶	FORMER OUTFALL
ORTF	OVER THE RIVER TANK FARM

NOTE:

LOCATIONS SHOWN ARE APPROXIMATE. THE LOCATIONS WILL BE FINALIZED BASED ON INFORMATION OBTAINED DURING THE CONDUCT OF THE HYDROLOGIC STUDY.

BASE MAP SOURCE:

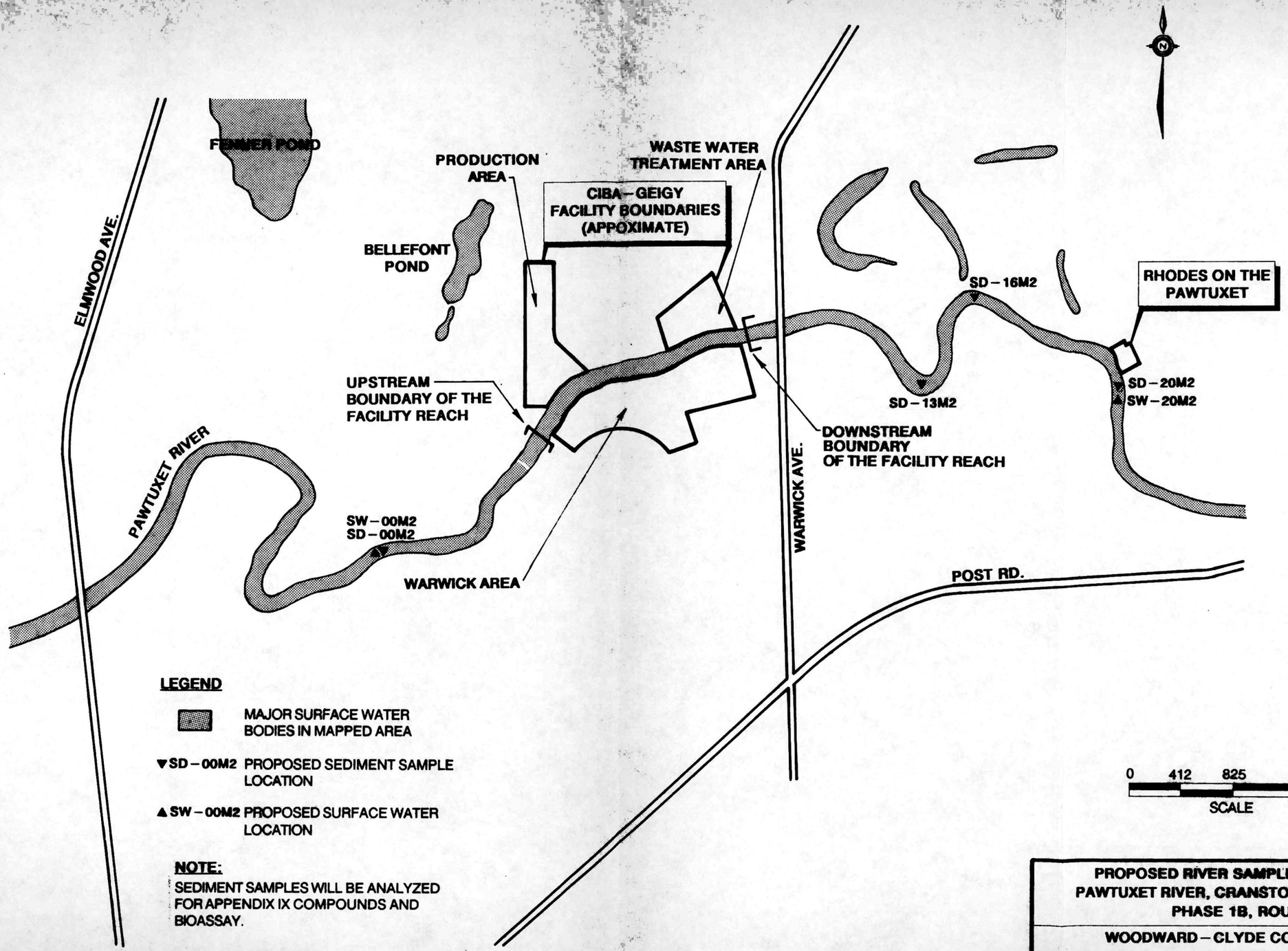
GEOD CORPORATION OF NEWFOUNDLAND, NEW JERSEY. MAPPED BY PHOTOGRAMMETRIC METHODS FROM AERIAL PHOTOGRAPHS. DATE FLOWN: 2 APRIL 1989.

SURFACE WATER AND SEDIMENT SAMPLING LOCATIONS PHASE IB, ROUND 1 CIBA – GEIGY FACILITY CRANSTON, RHODE ISLAND

WOODWARD – CLYDE CONSULTANTS

CONSULTING ENGINEERS, GEOLOGISTS AND ENVIRONMENTAL SCIENTISTS
WAYNE, NEW JERSEY

DR. BY: KF	SCALE: 1 : 2400	PROJ. NO.: 87X4660
CK'D. BY: PN	DATE: 19 SEPT 1990	FIG. NO.: 2



LEGEND

MAJOR SURFACE WATER BODIES IN MAPPED AREA

▼SD – 00M2 PROPOSED SEDIMENT SAMPLE LOCATION

▲SW – 00M2 PROPOSED SURFACE WATER LOCATION

NOTE:

SEDIMENT SAMPLES WILL BE ANALYZED FOR APPENDIX IX COMPOUNDS AND BIOASSAY.

BASE MAP SOURCE:

AERIAL PHOTOGRAPHS BY GEOD CORPORATION OF NEWFOUNDLAND, NEW JERSEY.
DATE FLOWN: 2 APRIL 1989.

PROPOSED RIVER SAMPLING LOCATIONS
PAWTUXET RIVER, CRANSTON, RHODE ISLAND
PHASE 1B, ROUND 2

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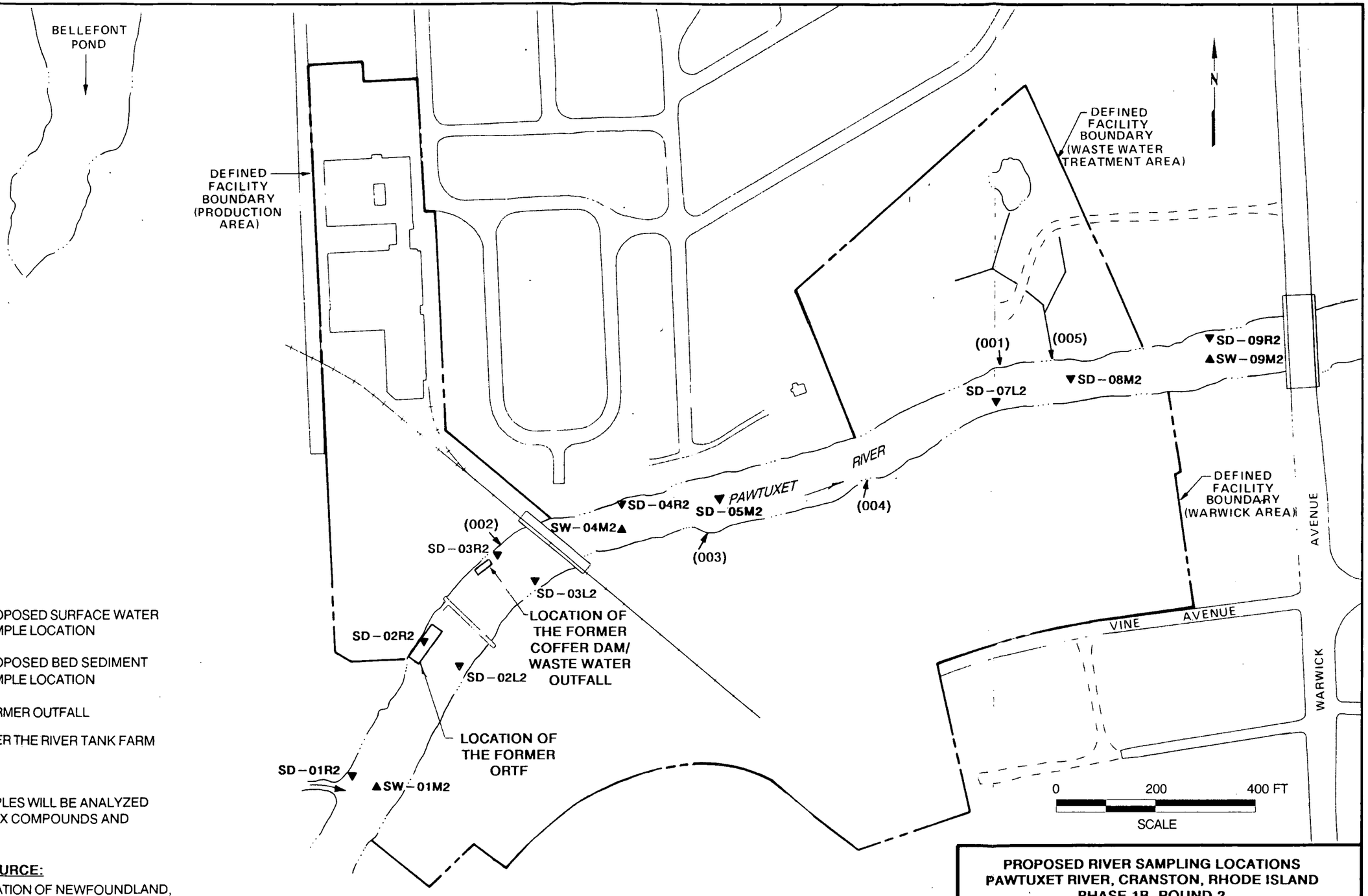
CONSULTING ENGINEERS, GEOLOGISTS AND ENVIRONMENTAL SCIENTISTS
WAYNE, NEW JERSEY

DR. BY: DRS	SCALE: 1 IN. ≃ 825 FT	PROJ. NO.: 87X4660
CK'D BY: MD	DATE: 27 FEB 1991	FIG. NO.: 3



LEGEND

▲ **SW – 01M2** PROPOSED SURFACE WATER SAMPLE LOCATION

▼ **SD – 01R2** PROPOSED BED SEDIMENT SAMPLE LOCATION

(001) FORMER OUTFALL

OTRF OVER THE RIVER TANK FARM

NOTE:

SEDIMENT SAMPLES WILL BE ANALYZED FOR APPENDIX IX COMPOUNDS AND BIOASSAY.

BASE MAP SOURCE:

GEOD CORPORATION OF NEWFOUNDLAND, NEW JERSEY. MAPPED BY PHOTOGRAMMETRIC METHODS FROM AERIAL PHOTOGRAPHS. DATE FLOWN: 2 APRIL 1989.

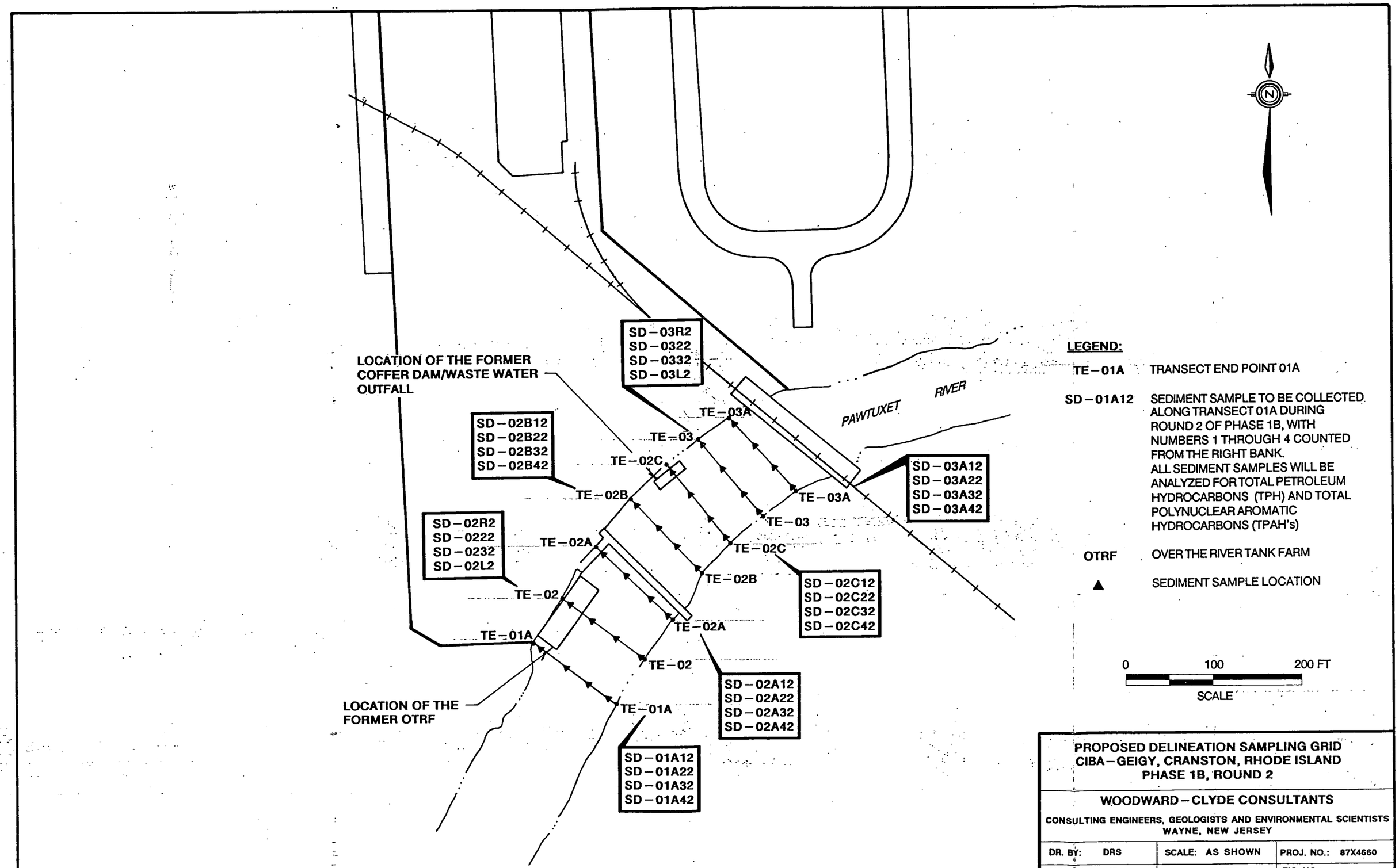
PROPOSED RIVER SAMPLING LOCATIONS
PAWTUXET RIVER, CRANSTON, RHODE ISLAND
PHASE 1B, ROUND 2

WOODWARD – CLYDE CONSULTANTS

CONSULTING ENGINEERS, GEOLOGISTS AND ENVIRONMENTAL SCIENTISTS
WAYNE, NEW JERSEY

DR. BY:	DRS	SCALE:	AS SHOWN	PROJ. NO.:	87X4660
CK'D BY:	MD	DATE:	28 FEB 1991	FIG. NO.:	4

REVISED SEPT. 7, 1990



PROPOSED DELINEATION SAMPLING GRID
CIBA – GEIGY, CRANSTON, RHODE ISLAND
PHASE 1B, ROUND 2

WOODWARD – CLYDE CONSULTANTS

CONSULTING ENGINEERS, GEOLOGISTS AND ENVIRONMENTAL SCIENTISTS
WAYNE, NEW JERSEY

DR. BY: DRS	SCALE: AS SHOWN	PROJ. NO.: 87X4660
CK'D BY: MD	DATE: 28 FEB 1991	FIG. NO.: 5

BIOASSAY OF THE TOXICITY OF SEDIMENTS, PORE WATERS, AND SURFACE WATERS COLLECTED FROM THE PAWTUXET RIVER NEAR THE FORMER FACILITY OF CIBA-GEIGY CORPORATION AT CRANSTON, RHODE ISLAND

Prepared by:

IT Corporation
Knoxville, Tennessee

MARCH 1991

CONTENTS

APPENDICES

TABLES

FIGURES

Number

ACRONYMS

AOC	Area of Concern
AAOI	Additional Area of Investigation
EPA	U.S. Environmental Protection Agency
IT	IT Corporation
PA	Production Area
PVC	Polyvinyl chloride
RCRA	Resource Conservation and Recovery Act
RFI	RCRA Facility Investigation
RVR	Round Valley Reservoir
SRC	Spruce Run Creek
SWMU	Solid Waste Management Unit
WA	Warwick Area
WWTA	Wastewater Treatment Area

EXECUTIVE SUMMARY

A part of Phase I of the Resource Conservation and Recovery Act (RCRA) Facility Investigation (RFI) being undertaken at the former CIBA-GEIGY Corporation (CIBA-GEIGY) manufacturing facility in Cranston, Rhode Island, is the Public Health and Environmental Risk Evaluation (PHERE). The primary focus of the environmental receptor investigation portion of the PHERE is the characterization of the impact on biota in the Pawtuxet River. The river biota possess the greatest potential for environmental impact due to possible releases from Solid Waste Management Units (SWMUs) and Areas of Concern (AOCs). A Screening Level Assessment, consisting of a series of bioassays, was used for this characterization.

The media of the Pawtuxet River investigated in the Screening Level Assessment included surface water, sediment, and interstitial pore water. The chronic toxicity of surface water was evaluated using the fathead minnow, *Pimephales promelas*, and the water flea, *Ceriodaphnia dubia*. The sediments were evaluated for acute toxicity of the sediment with its pore water to the midge larvae, *Chironomus tentans*, and for acute toxicity of the pore water to the water flea, *Ceriodaphnia dubia*.

Ten sampling stations were identified in the region of the former CIBA-GEIGY Corporation facility: two stations upstream of the site, two stations downstream of the site, and six stations along the facility reach. The six site stations were chosen for their proximity to facility outfalls and past releases. Surface water was sampled and tested at both upstream stations, both downstream stations, and two of the six site stations. Sediment was sampled and tested at all ten stations. Pore water was tested for all ten sediment samples.

Sediment sample consistency ranged from coarse sand at the far upstream station and both downstream stations, to medium sand at the station just off the residential section in the middle of the site, to silt and silt/clay at all stations off the Production Area or

Wastewater Treatment Area.

Surface water samples showed no toxicity. Survival and growth of *Pimephales promelas* and survival and reproduction of *Ceriodaphnia dubia* exposed to site samples were not significantly different from that in the reference samples.

The results of these screening bioasays showed that four of the six CIBA-GEIGY sediment samples along the facility reach resulted in complete mortality to *Chironomus tentans*. The toxicity of these samples was greater than that of the Pawtuxet River system upstream or downstream of the site. The other two site samples, though showing less survival than either the laboratory control or the far upstream station, did not result in significant differences from the near upstream station or the two downstream stations. Toxicity in these samples could also be due to the general nature of the impacted Pawtuxet River system. Survival of chironomids exposed to laboratory sediment in the 10-day acute test was 60 percent. This is less than the 80 or greater percent that is normally expected for the reference sediment. This observation may cast doubt on the interpretation of the bioassay.

Two of the six site sediment samples contained pore water acutely toxic to *Ceriodaphnia dubia*. These samples were taken from the region of the Coffer Dam/Wastewater Outfall and the Wastewater Treatment Area.

1.0 INTRODUCTION

The test results reported here are part of Phase I of the Resource Conservation and Recovery Act (RCRA) Facility Investigation (RFI) being undertaken at the former CIBA-GEIGY Corporation (CIBA-GEIGY) manufacturing facility in Cranston, Rhode Island (the site).

1.1 SITE DESCRIPTION AND HISTORY

The former CIBA-GEIGY manufacturing facility is located in the urban communities of Cranston and Warwick, Rhode Island. The site is divided into three areas: the Production Area, the Wastewater Treatment Area, and the Warwick Area. The first two areas are north of the Pawtuxet River. The Warwick Area is south of the river.

Twelve Solid Waste Management Units (SWMUs), two Areas of Concern (AOCs), and two Additional Areas of Investigation (AAOIs) have been identified as locations of former production facilities, waste treatment or waste storage sites, locations of documented spills, or areas of historical releases of hazardous substances (Table 1).

1.2 PURPOSE OF BIOASSAY TESTING

This bioassay testing was conducted as part of Phase I of the RFI to aid in identifying toxic characteristics of water and sediment in the Pawtuxet River.

1.3 APPROACH

Chemical contamination could result in toxicants residing in the solid matter of the river sediment, within the interstitial waters of the sediment (pore water), or in the surface waters of the river. Therefore, these three media were used in bioassay testing.

Three species were used for testing. The fathead minnow, *Pimephales promelas,* was used in testing the toxicity of surface waters. *Pimephales promelas* is a surface water fish with a widespread distribution throughout the United States. This species represents an

Table 1

Solid Waste Management Units, Areas of Concern, and

Additional Areas of Investigation

Area	Description	Location
SWMU-1 [a]	Hazardous waste storage area	WA [b]
SWMU-2	Hazardous waste storage area	PA [c]
SWMU-3	Hazardous waste storage area	PA
SWMU-4	Trash compactor station	PA
SWMU-5	River sediment storage area	WA
SWMU-6	Zinc oxide/soil pile	WA
SWMU-7	Chlorosulfonic acid spill area	PA
SWMU-8	Prussian Blue spill area	PA
SWMU-9	Wastewater pipeline break	WA
SWMU-10	Wastewater pipeline break	WWTA [d]
SWMU-11	Toluene waste water release	PA
SWMU-12	Waste water treatment area	WWTA
AOC-13 [e]	Process building	PA
AOC-14	Atlantic Tubing and Rubber Company	West of PA
AAOI-15 [f]	Laboratory building waste water sump	North of PA
AAOI-16	Maintenance department cleaning area	WA

a SWMU - Solid Waste Management Unit.
b WA - Warwick Area.
c PA - Production Area.
d WWTA - Wastewater Treatment Area.
e AOC - Area of Concern.
f AAOI - Additional Area of Investigation.

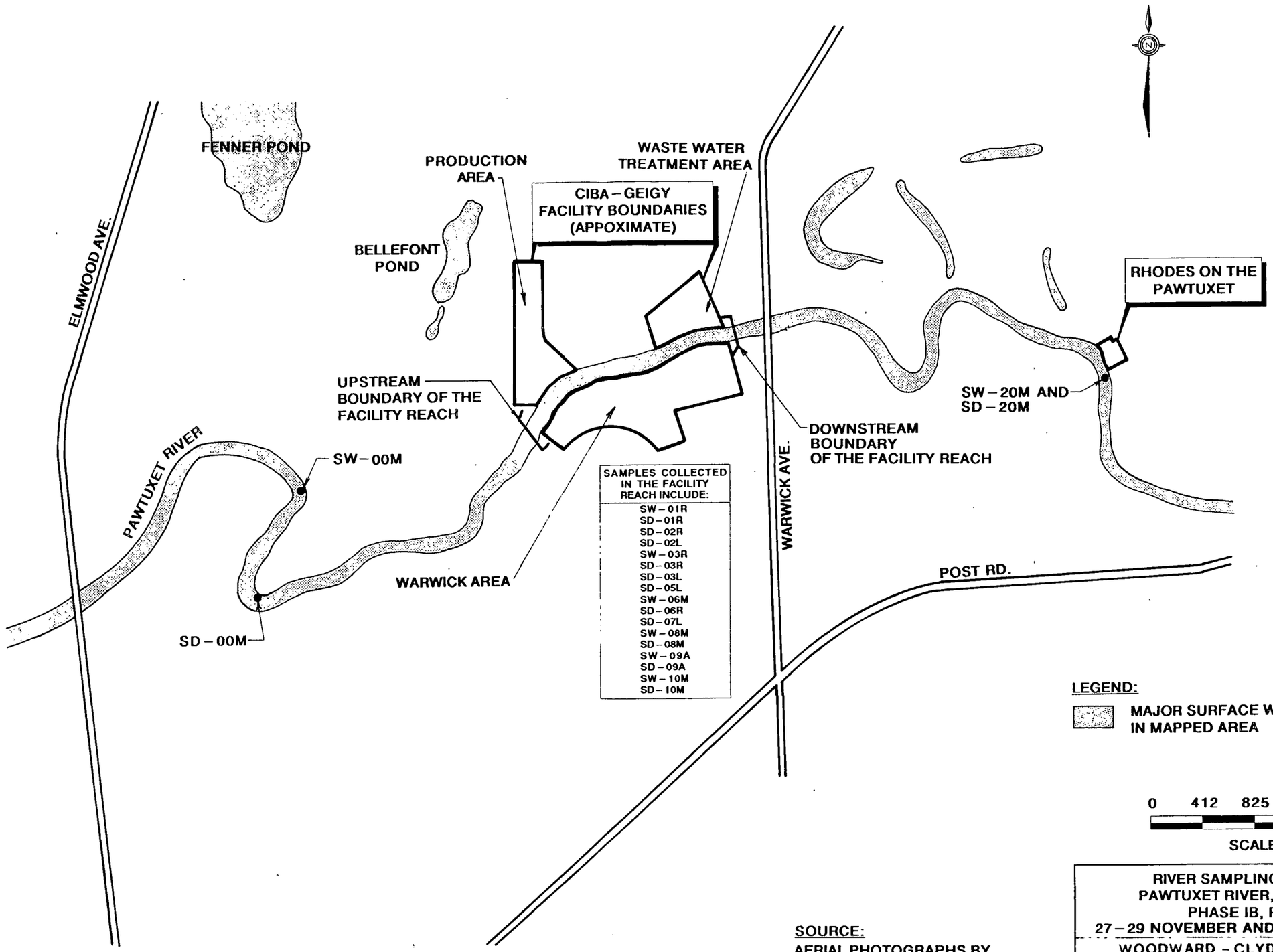
important forage fish in the food chain, and it is readily cultured in the laboratory (Norberg and Mount, 1985). The water flea, *Ceriodaphnia dubia*, was employed in bioassays of surface water and pore water. *Ceriodaphnia dubia* is a small, planktonic, freshwater crustacean, commonly found in lakes and large rivers throughout the world. It plays an important role as a herbivore, feeding on algae, and as prey for many vertebrate species, particularly fish. These characteristics plus their relatively short life-cycle make *Ceriodaphnia dubia* a useful test organism for measuring aquatic toxicity. Second instar larvae of the midge, *Chironomus tentans*, were used in sediment testing. *Chironomus tentans* are detritivores that live in sediment, thereby being exposed to toxicants through feeding and contact with the sediment.

2.0 METHODS

The media of the Pawtuxet River investigated in the Screening Level Assessment were surface water, sediment, and interstitial pore water. The chronic toxicity of surface water was evaluated using the fathead minnow, *Pimephales promelas*, and the water flea, *Ceriodaphnia dubia*. The sediments were evaluated for acute toxicity of the sediment itself with larvae of the the midge, *Chironomus tentans*, and for acute toxicity of the interstitial pore waters of the sediments with *Ceriodaphnia dubia*.

Ten sampling stations were identified in the region of the former CIBA-GEIGY facility: two stations upstream of the site, two stations downstream of the site, and six stations along the facility reach. The six site stations were chosen for their proximity to facility outfalls and past releases. Surface water was sampled and tested at both upstream stations, both downstream stations, and two of the six site stations. Sediment was sampled and tested at all ten stations. Pore water was tested for all ten sediment samples. Sampling locations are shown in Figures 1 and 2.

Detailed descriptions of the culturing of test organisms, sample collection and handling, bioassay test methodology, and statistical methods used in analyzing the test results are

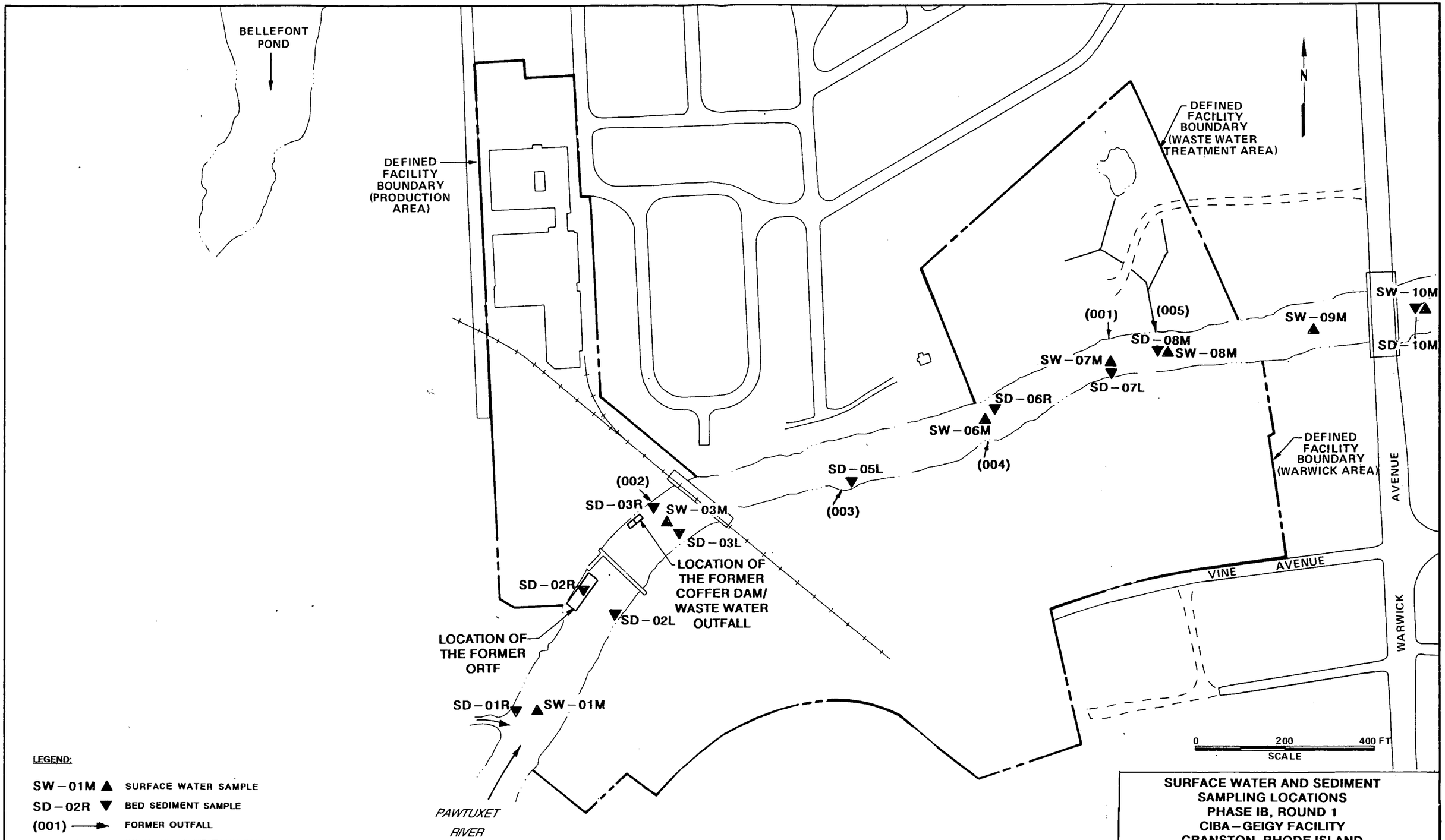


SOURCE:
AERIAL PHOTOGRAPHS BY GEOD CORPORATION OF NEWFOUNDLAND, NEW JERSEY.
DATE FLOWN: 2 APRIL 1989

RIVER SAMPLING LOCATIONS
PAWTUXET RIVER, CRANSTON, RI
PHASE IB, ROUND 1
27 – 29 NOVEMBER AND 7 DECEMBER, 1990

WOODWARD – CLYDE CONSULTANTS
CONSULTING ENGINEERS, GEOLOGISTS AND ENVIRONMENTAL SCIENTISTS
WAYNE, NEW JERSEY

DR.	MG	1" ≅ 825 FEET	87X4660
APP.	MEPD	12 FEB 1991	1



LEGEND:

SW – 01M ▲	SURFACE WATER SAMPLE
SD – 02R ▼	BED SEDIMENT SAMPLE
(001) ⟶	FORMER OUTFALL
ORTF	OVER THE RIVER TANK FARM

NOTE:

LOCATIONS SHOWN ARE APPROXIMATE. THE LOCATIONS WILL BE FINALIZED BASED ON INFORMATION OBTAINED DURING THE CONDUCT OF THE HYDROLOGIC STUDY.

BASE MAP SOURCE:

GEOD CORPORATION OF NEWFOUNDLAND, NEW JERSEY. MAPPED BY PHOTOGRAMMETRIC METHODS FROM AERIAL PHOTOGRAPHS. DATE FLOWN: 2 APRIL 1989.

SURFACE WATER AND SEDIMENT SAMPLING LOCATIONS PHASE IB, ROUND 1 CIBA – GEIGY FACILITY CRANSTON, RHODE ISLAND

WOODWARD – CLYDE CONSULTANTS

CONSULTING ENGINEERS, GEOLOGISTS AND ENVIRONMENTAL SCIENTISTS
WAYNE, NEW JERSEY

DR. BY: KF	SCALE: 1 : 2400	PROJ. NO.: 87X4660
CK'D. BY: PN	DATE: 19 SEPT 1990	FIG. NO.: 2

presented in Appendix A. Standard Operating Procedures (SOPs) for bioassay tests are presented in Appendix B.

3.0 RESULTS

3.1 THREE BROOD CHRONIC BIOASSAY OF SURFACE WATERS USING *Ceriodaphnia dubia*

Results are shown in Table 2. Survival of test organisms was at least 90 percent for all test samples and controls. Average neonate production per surviving female ranged from 6.6 for the near downstream sample (SW-10M), to 17.7 for the far upstream sample (SW-00M) during the first set of tests. Neither of the samples collected from the CIBA-GEIGY site (SW-03M and SW-07M) was associated with a significant decrease in reproduction or survival, compared with the reference samples or the laboratory control. The average numbers of neonates per adult in both upstream reference samples (SW-00M and SW-01M) were significantly lower than the laboratory control in the second test. Average neonates per adult were significantly less in the near upstream sample (SW-01M) than in the far upstream sample (SW-00M). Average neonates per adult in both downstream samples (SW-10M and SW-20M) were significantly less than both the laboratory controls and far upstream samples (SW-00M). Reproduction as measured by average neonates per adult in the near downstream sample (SW-10M) was also significantly less than that seen in the near upstream sample (SW-01M).

3.2 SEVEN-DAY CHRONIC BIOASSAY OF SURFACE WATERS USING *Pimephales promelas*

Results are shown in Table 3. Survival of test organisms was at least 87.5 percent for all test samples and controls. Mean dry weights per organism ranged from 0.777 to 1.011 milligrams (mg) during the first test and from 0.561 to 0.682 mg during the second test. No significant differences were observed in survival or growth of exposed organisms for any test samples compared with the laboratory controls or reference samples.

Table 2

Three Brood Chronic Bioassay of Surface Water Samples Collected Near the Former CIBA-GEIGY Facility at Cranston, Rhode Island, Using the Water Flea, *Ceriodaphnia dubia*

Sample	Percent Survival and Reproduction			
	Test start 11/29/90		Test start 12/8/90	
	Survival (%) (n=10)	Neonates per adult	Survival (%) (n=10)	Neonates per adult
Laboratory water	90	17 [a] (1.69)	100	16.7 [a] (2.50)
SW-00M	100	17.7 (1.25)	100	12.6 [b] (2.01)
SW-01M	100	14.9 [c] (2.15)	100	14.2 [b] (3.12)
SW-03M	100	17.6 (2.32)	NA	NA
SW-07M	100	14.6 (5.40)	NA	NA
SW-10M	NA	NA	100	6.6 [b,c,d] (1.96)
SW-20M	90	13.0 [b,c] (2.78)	NA	NA

a Arithmetic means, standard deviations in parentheses.
b Significantly different from laboratory water.
c Significantly different from SW-00M.
d Significantly different from SW-01M.

NA - Not applicable, test not performed.

Table 3

Seven-Day Chronic Bioassay of Surface Water Samples Collected Near the Former CIBA-GEIGY Facility at Cranston, Rhode Island, Using the Fathead Minnow, *Pimephales promelas*

Sample	Percent Survival and Final Dry Weight			
	Test start 11/29/90		Test start 12/8/90	
	Mean Survival [a] (%)	Mean Dry Weight [b] (mg)	Mean Survival [a] (%)	Mean Dry Weight [b] (mg)
Laboratory water	90 (0.82)	0.959 (0.046)	100 (0.0)	0.664 (0.083)
SW-00M	92.5 (5.0)	0.891 (0.105)	90 (0.0)	0.633 (0.068)
SW-01M	100 (0.0)	0.777 (0.155)	95 (5.8)	0.561 (0.168)
SW-03M	92.5 (5.0)	1.011 (0.151)	NA	NA
SW-07M	87.5 (5.0)	0.897 (0.063)	NA	NA
SW-10M	NA	NA	90 (8.2)	0.682 (0.126)
SW-20M	97.5 (5.0)	0.825 (0.076)	NA	NA

a Arithmetic mean of 4 tanks with 10 fish/tank (standard deviations in parentheses).
b Arithmetic mean of 4 tanks; fish not weighed individually (standard deviations in parentheses).
NA - Not applicable, test not performed.

3.3 FORTY-EIGHT-HOUR ACUTE BIOASSAY OF PORE WATERS USING *Ceriodaphnia dubia*

Results are shown in Table 4. Survival of test organisms was at least 87.5 percent for all but two samples tested. All test organisms exposed to pore water from sample SD-03R were dead at 24 hours. Only 15 percent of organisms exposed to pore water from sample SD-06R survived to 48 hours. Survival of daphnias for samples SD-03R and SD-06R was found to be significantly less than that for the laboratory control or either upstream reference sample (SD-00M and SD-01M).

3.4 TEN-DAY ACUTE BIOASSAY OF SEDIMENTS USING *Chironomus tentans*

Results are shown in Table 5. Survival of chironomids exposed to the laboratory control sediment was 60 percent, whereas 80 percent would normally be expected. Survival among test organisms exposed to the far upstream sample (SD-00M) was 65 percent, which exceeded the survival seen in all other river samples. Survival in the near upstream sample (SD-01R) was only 33.8 percent. Total mortality of test organisms occurred at four of the six CIBA-GEIGY locations tested: SD-02R, SD-03R, SD-06R, and SD-08M. Survival in samples SD-05L and SD-07L was 32.5 and 20 percent, respectively, which did not differ significantly from the near upstream control (SD-01R). Survival in the downstream samples (SD-10M and SD-20M) was 28.8 and 3.75 percent, respectively. However, only survival in samples SD-02R, SD-03R, SD-06R, SD-08M, and SD-20M was significantly less than that in the near upstream sample (SD-01R).

4.0 DISCUSSION

Results of surface water testing using both the *Ceriodaphnia dubia* three-brood chronic test and the *Pimephales promelas* seven-day chronic test on surface water samples indicated no toxicity in the surface waters of the Pawtuxet River in the region of the former CIBA-GEIGY facility.

Survival of chironomids exposed to laboratory sediment in the 10-day acute test was 60

Table 4

Forty-Eight-Hour Acute Bioassay of Pore Water from Sediment Samples Collected Near the Former CIBA-GEIGY Facility at Cranston, Rhode Island, Using the Water Flea, *Ceriodaphnia dubia*

Sample Location	Percent Survival	
	Arithmetic Mean n = 4 chambers 10 organisms/chamber	Standard Deviation
Reference Sediment	90.0	8.2
SD-00M	97.5	5.0
SD-01R	97.5	5.0
SD-02R	100	0
SD-03R	0 [a,b,c]	0
SD-05L	87.5	9.6
SD-06R	15.0 [a,b,c]	12.9
SD-07L	100	0
SD-08M	100	0
SD-10M	97.5	5.0
SD-20M	100	0

a Significantly different from reference sediment.
b Significantly different from SD-00M.
c Significantly different from SD-01R.

Table 5

Ten-Day Acute Bioassay of Sediment Collected near the Former CIBA-GEIGY Facility at Cranston, Rhode Island, Using the Midge Larvae, *Chironomus tentans*

Station Location	Percent Survival	
	Arithmetic Mean n = 4 chambers 20 organisms/chamber	Standard Deviation
Reference Sediment	60.0	10.8
SD-00M	65.0	23.8
SD-01R	33.8	18.9
SD-02R	0 [a,b,c]	0
SD-03R	0 [a,b,c]	0
SD-05L	32.5 [b]	27.2
SD-06R	0 [a,b,c]	0
SD-07L	20.0 [a,b]	10.8
SD-08M	0 [a,b,c]	0
SD-10M	28.8 [b]	8.5
SD-20M	3.75 [a,b,c]	4.8

a Significantly different from reference sediment.
b Significantly different from SD-00M.
c Significantly different from SD-01R.

percent. This is less than the 80 or greater percent that is normally expected for the reference sediment. However, this somewhat lowered survival in the laboratory control does cast doubt on the interpretation of the bioassay. Nevertheless, it does appear that some river sediments collected along the facility reach are more toxic than the laboratory control sediments. Four of the six CIBA-GEIGY site samples showed 100 percent mortality. Three of these (SD-02R, SD-03R, and SD-06R) were collected near the north bank of the river and the other, SD-08M, was taken from north of midstream. The two samples of sediment taken from the south bank of the river (SD-05L and SD-07L) did not differ in toxicity from the near upstream sample (SD-01R).

Results of the 48-hour acute tests on the pore water samples using *Ceriodaphnia dubia* differed somewhat from the results with sediment. Two of the four locations at the CIBA-GEIGY site that demonstrated toxicity in sediment showed significant toxicity in pore waters. This suggests that some portion of the toxicants in sediment resides in the interstitial waters and is not tightly bound to the sediment itself. However, the lack of toxicity in the water column itself suggests that such toxicity might be localized to the benthos. The lack of toxicity to *Ceriodaphnia dubia* in pore waters from the other sediment samples is important because it indicates that toxicants at these locations might be tightly bound to sediments.

5.0 CONCLUSIONS

1. These results show evidence of toxicity in the sediments of the Pawtuxet River. The toxicity appears to be limited to the sediments and interstitial waters only. The most sensitive organism for detecting this toxicity is *Chironomus tentans*.

2. The full extent of the impact on the sediments cannot be determined without further toxicological examinations. Disturbance of sediments caused by remedial activity could cause release of toxicants within the interstitial waters in toxic amounts. Further toxicity testing could aid in evaluating this possibility.

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APPENDIX A

DESCRIPTION OF METHODS

APPENDIX A

CONTENTS

TABLES

APPENDIX A
DESCRIPTION OF METHODS

1.0 CULTURING OF TEST ORGANISMS

All organisms used in toxicity tests were cultured at IT Corporation's bioassay laboratory in Edison, New Jersey. Continuous culturing in-house allows the bioassay laboratory to monitor and document the life history of the test organisms and the cultures from which they are obtained and to provide a ready and consistent test species.

1.1 WATER FLEAS

Stock cultures of water fleas (*Ceriodaphnia dubia*) are routinely kept at IT's bioassay laboratory. Cultures are maintained according to U.S. Environmental Protection Agency (EPA) guidelines (Peltier and Weber, 1985). Stock cultures consisted of daphnids fed the green alga, *Selenastrum capricornutum,* in accordance with Goulden and Henry (1984) and Cowgill *et al.* (1984). Ceriodaphnids were cultured in filtered water from Round Valley Reservoir (RVR) (Goulden and Henry, 1984). RVR water is used as the culture water at the bioassay laboratory because nutrients, pH, and hardness are at optimal levels for culturing daphnids. *Ceriodaphnia dubia* was maintained in this water until the initiation of testing. At 24 hours before the initiation of testing, all neonates were removed from the cultures and discarded. Therefore, all neonates drawn from the cultures just before test initiation were less than 24 hours old.

1.2 FATHEAD MINNOWS

Fathead minnows (*Pimephales promelas*) used for bioassay testing were cultured at IT's bioassay laboratory in accordance with Denny (1987). Breeding-age fish were maintained in 20-gallon glass aquaria containing RVR water. Breeding aquaria were serviced by a recirculating water, carbon treatment, and filtration system. Eggs laid on the underside of polyvinyl chloride (PVC) huts were removed from the tanks daily. Huts were transferred to hatching trays in which a few drops of methylene blue were added to the water to prevent fungal growth. The eggs were allowed to hatch, and the larvae

were monitored for excessive mortality or unusual behavior.

1.3 MIDGES

Midge larvae (*Chironomus tentans*) used for bioassay testing were cultured at IT's bioassay laboratory following the procedure of Nebecker et al. (1984). Midge cultures were contained within 10-gallon glass aquaria aerated by oil-free air lines and maintained at 24±2°C. Weekly renewals of water were made with moderately hard reconstituted water (Peltier and Weber, 1985) to which crushed paper towels were added, as needed, to act as a substrate. Animals were fed *ad libitum* on a diet consisting of pulverized cereal leaves and Tetramin® flakes. Adult midges were removed from the culture aquaria using an aspirator and were placed in a 5-liter (L) glass breeder aquarium containing moderately hard reconstituted water, cereal leaves, and cultured *Selenastrum capricornutum*. Larvae were maintained within this aquarium until the initiation of testing.

2.0 SAMPLE COLLECTION AND HANDLING

Collection of surface waters and sediments at the site was conducted by personnel from Normandeau Associates, Inc., Woodward-Clyde Consultants, and IT. Dates for surface water and sediment samples are listed in Table A-1.

2.1 SELECTION OF SAMPLING SITES

All stations for collection of surface waters and sediments were located in the Pawtuxet River in Cranston or Warwick, Rhode Island, through an area ranging from approximately 1.5 miles upstream of the site to approximately 1.5 miles downstream of the site. Surface waters and sediments were collected where the river runs through the site, upstream from the site, and downstream from the site. Samples at the site were collected at points where maximum concentrations of toxicants might have been expected, due either to activities at the site or to the dynamics of the river. Samples upstream and downstream from the site were selected because they were at pronounced meander bends or at other areas likely to afford sediment. Both Woodward-Clyde

Table A-1

Dates of Collection of Bioassay Samples of Surface Water and Sediment from the Pawtuxet River in the Vicinity of the Former CIBA-GEIGY Facility at Cranston, Rhode Island

Collection Period	IT Location Code	Woodward-Clyde Location Code	
		Surface Water	Sediment
November 27-29, 199	0U-1	SW-00M	SD-00M
	U-2	SW-01M	SD-01R
	CG-3	NA	SD-02R
	CG-4	SW-03M	SD-03R
	CG-5	NA	SD-05L
	CG-6	NA	SD-06R
	CG-7	SW-07M	SD-07L
	CG-8	NA	SD-08M
	D-9	SW-20M	SD-20M
December 7, 1990	U-1	SW-00M	NA
	U-2	SW-01M	NA
	D-9A	SW-10M	SD-10M

NA - Not applicable, no sample collected.

Consultants and IT sample designations are given in Table A-1. Approximate locations of sampling points are shown in Figures 1 and 2.

2.2 DESCRIPTION OF THE SAMPLING LOCATIONS

Stations U1 and U2 are both upstream of the site. These were chosen as reference stations outside of and before any influence by the site. Station U1 is located approximately 1.5 miles upstream of the site at a series of sharp bends in the Pawtuxet River near the intersections of Moore Avenue and Youlden Avenue in Cranston. Samples of surface water (SW-00M) and sediment (SD-00M) were collected near midstream at U1. Station U2 was located immediately upstream of the site and just downstream from the confluence of the Pawtuxet River with a creek that flows south from Bellefont Pond. Station U2 is close to the Atlantic Tubing and Rubber Company. Surface water was collected near midstream (SW-01M), and sediment was collected near the north bank of the river (SD-01R).

Stations CG3 and CG4 are located in the Pawtuxet River, off the Production Area and across the river from the western portion of the Warwick Area. Station CG3 is located in the vicinity of a former over-the-river tank farm, just downstream of the western extent of the Production Area. Sediment was collected near the north bank (SD-02R). Station CG4 is located immediately downstream of the location of the former Coffer Dam and wastewater outfall, just upstream of the eastern extent of the Production Area, near the automobile and railroad bridge connecting the Production Area and the Warwick Area. Surface water was collected near the middle of the river (SW-03M), and sediment was collected near the north bank (SD-03R).

Station CG5 is located in the Pawtuxet River off the midportion of the Warwick Area, across the river from the foot of Mayflower Drive. Sediment was collected near the south bank of the river (SD-05L).

Stations CG6, CG7, and CG8 are located in the Pawtuxet River off the Wastewater Treatment Area and the eastern portion of the Warwick Area. Sediment was collected at

Station CG6 just north of midstream (SD-06R), immediately downstream of the western boundary of the Wastewater Treatment Area. Station CG7 is located on the Warwick side, across from the middle portion of the Wastewater Treatment Area. Surface water was collected at midstream (SW-07M) and sediment was collected near the south bank (SD-07L). Station CG8 is located just upstream of the eastern extent of both the Wastewater Treatment Area. Sediment was collected north of midstream (SD-08M).

Stations D9A and D9 are located downstream of the former CIBA-GEIGY facility. Station D9A is just downstream of the Warwick Avenue bridge, approximately 1/4 mile downstream of the site. This station was added to the sampling plan after the initial sampling effort was conducted. Surface water and sediment were collected near midstream (SW-10M, SD-10M). Station D9 is located approximately 1.5 miles downstream of the site at a sharp bend in the river just downstream of Rhodes on the Pawtuxet, a location where the river becomes wider and shallower than at the other sampling locations. Surface water and sediment were collected near midstream (SW-20M, SD-20M).

2.3 SAMPLE COLLECTION

Samples were collected in two events. The first sampling event took place during the week of November 26, 1990. By the following week, it had been decided to collect samples from an additional downstream point closer to the site. Because bioassays of surface water had already begun, the second sampling event included resampling of surface waters at upstream and downstream reference points. The two events are detailed in Table A-1 and Figures 1 and 2.

Samples of surface water were obtained as multiple grabs made with a polypropylene beaker. These were composited into a 5-gallon polyethylene Cubitainer®. A minimum of 2.5 gallons was collected for each sample of surface water. Cubitainers were packed in ice during transport to the bioassay laboratory.

A Ponar Grab Sampler was used to collect samples of sediment. Multiple grabs (minimum

of two) were necessary to provide sufficient sediment of appropriate size and sufficient pore water. Sediment samples were placed in 7-gallon polyethylene bags within 5-gallon polypropylene buckets. The bags were sealed and maintained on ice during transport to the bioassay laboratory. All samples were stored at the bioassay laboratory in refrigerators maintained at approximately 4°C.

2.4 SAMPLE APPEARANCE

The sediment samples collected ranged in consistency from coarse sand to silt and silt/clay (Table A-2). The far upstream sample (SD-00M), the two downstream samples (SD-10M and SD-20M), and the furthest downstream site sample (SD-08M) were coarse sand. The sample collected off the Warwick Area across from the residential section between the Production Area and the Wastewater Treatment Area (SD-05L) was medium sand. The remaining site samples (SD-02R, SD-03R, SD-06R, and SD-07L) and the near upstream sample (SD-01R) ranged in consistency from sand/silt to silt/clay.

2.5 SAMPLE HANDLING AND PREPARATION

Surface waters were allowed to warm to room temperature before testing. Samples were then passed through a 60 μm plankton net to remove debris or indigenous organisms (Weber et al., 1989).

Sediments were removed from cold storage on December 12, 1990, and all waters over the surface of the sediments were decanted. The sediments were then stirred with a PVC rod and aliquots of approximately 40 ml were removed. These were placed in disposable 50-milliliter (ml) polypropylene centrifuge tubes and centrifuged at 20,000 revolutions per minute (rpm) for 20 minutes in a Sorvall Superspeed Model RC2-B refrigerated centrifuge with a Sorvall Instruments SS-34 rotor. The extracted interstitial waters were then poured off and collected in 500-ml polypropylene bottles. This process was repeated until at least 400 ml of pore water was obtained from each of the 10 sediment samples. Each pore water sample was then vacuum filtered through a 0.45 um glass microfiber filter to remove suspended solids that could confound the bioassay by interfering with ceriodaphnid

Table A-2

Physical Description of Sediment Samples Collected near the Former CIBA-GEIGY Facility at Cranston, Rhode Island

Sample	Physical Description
SD-00M	Coarse sand
SD-01R	Sand/silt
SD-02R	Silt
SD-03R	Silt/clay
SD-05L	Medium sand
SD-06R	Silt/sand
SD-07L	Silt/clay
SD-08M	Coarse sand
SD-10M	Coarse sand
SD-20M	Coarse sand

movement. Pore water samples were stored at approximately 4°C until ready for test initiation.

The remaining sediments were then filtered through an American Society for Testing and Materials (ASTM) Standard No. 18 sieve with 1-millimeter (mm) openings to remove large particles and endemic animals, especially predators. Sieving of sediments was performed without the introduction of additional water. Sieved sediment samples were stored in the dark at approximately 4°C until ready for the initiation of testing.

3.0 TEST METHODOLOGY

3.1 THREE BROOD CHRONIC TEST OF TOXICITY OF SURFACE WATERS USING *Ceriodaphnia dubia*

The three brood bioassays on the surface waters using *Ceriodaphnia dubia* were based on IT Testing SOP Standard Operating Procedure (SOP) A2.0, which is included in Appendix B. This assay follows recommended EPA test methodology (Weber et al., 1989). Two sets of *Ceriodaphnia dubia* three brood chronic tests were conducted, corresponding to the two sampling events. The dates of the initiation of these tests are given in Table A-3.

Ceriodaphnia dubia neonates used in testing were less than 24 hours old and born within a period of 8 hours. Test chambers were 30-ml polypropylene cups filled to a volume of 15 ml. Ten replicates for each surface water sample plus laboratory controls of RVR were prepared. One organism was introduced into each test chamber. Fresh solutions of filtered, undiluted river water were prepared daily in 30-ml cups, and the test organisms were transferred to the new solutions using a wide bore pipet. Daphnids were released below the water-air interface to reduce the risk of air being trapped under the carapace. Reproduction was measured at the end of each 24-hour exposure period by counting the neonates in the test solution after transferring the test organism. The temperature was maintained at 25 ± 1°C by placing test chambers in a styrofoam float

Table A-3

Test Initiation Dates for Bioassays Performed on Samples Collected near the Former CIBA-GEIGY Facility at Cranston, Rhode Island

Sample Code	Test Type			
	Ceriodaphnia dubia chronic test on surface waters	*Pimephales promelas* chronic test on surface waters	*Ceriodaphnia dubia* acute test on pore waters	*Chironomus tentans* acute test on sediments
SW-00M, SD-00M	11/29/90, 12/08/90	11/29/90, 12/08/90	12/13/90	12/13/90
SW-01M, SD-01R	11/29/90, 12/08/90	11/29/90, 12/08/90	12/13/90	12/13/90
SD-02R	NA	NA	12/13/90	12/13/90
SW-03M, SD-03R	11/29/90	11/29/90	12/13/90	12/13/90
SD-05L	NA	NA	12/13/90	12/13/90
SD-06R	NA	NA	12/13/90	12/13/90
SW-07M, SD-07L	11/29/90	11/29/90	12/13/90	12/13/90
SD-08M	NA	NA	12/13/90	12/13/90
SW-10M, SD-10M	12/08/90	12/08/90	12/13/90	12/13/90
SW-20M, SD-20M	11/29/90	11/29/90	12/13/90	12/13/90

NA - Not applicable; no test performed on surface water from this station.

in a temperature-controlled water bath (Forma Scientific). Laboratory lighting was maintained at 50 100-foot-candles in intensity with a photoperiod of 16-hour light and 8-hour dark. The test animals were fed daily with a unicellular green alga, *S. capricornutum*, to an initial density of 100,000 cells/ml. The tests were terminated after 6 days duration when at least 80 percent of surviving females in the control and reference samples (SW-00M and SW-01M) had produced their third broods. The test end points measured were survival and reproduction.

3.2 SEVEN-DAY CHRONIC TEST OF TOXICITY OF SURFACE WATERS USING *Pimephelas promelas*

The seven-day bioassays on the surface water using *Pimephelas promelas* were based on IT Testing SOP A10.0, which is included in Appendix B. This assay follows recommended EPA test methodology (Weber et al., 1989). Two sets of seven-day chronic tests were conducted using *Pimephelas promelas*, corresponding to the two sampling events. The dates of the initiation of these tests are given in Table A-3.

Fathead minnow fry less than 48 hours old and all within 24 hours of age were used in the 7-day chronic bioassays. Test chambers consisted of 1 L glass beakers filled to a volume of 400 ml. Four replicates for each surface water sample plus laboratory controls of RVR were prepared. Organisms were transferred randomly from hatching trays to nontoxic food-grade 2-fluid-ounce cups with a wide bore pipet. Ten organisms were randomly transferred to the test beakers by partially submerging the cups in the beakers to allow the fish to swim freely into the test solutions. Test beakers were placed randomly in the test area.

Following daily observations of survival and behavior, test solutions were slowly poured from the beakers to the minimum water level, which still allowed unstressed swimming by the fish (approximately 1 cm). Fresh solutions of undiluted, filtered river water were prepared and introduced to the test chambers by slowly pouring the test water down the side of the beaker to minimize the turbulence and stress to the test organisms. Minnows were fed equal amounts of newly hatched brine shrimp, *Artemia sp. nauplii*, twice daily to ensure adequate

food for survival and growth throughout the test period. Remaining food and wastes were removed from the beakers daily by gentle siphon with a disposable transfer pipet. After seven days of exposure, the tests were ended and the dry weight of the surviving fish determined. Individual weights were not collected for each fish; rather, the total dry weight of fish in each beaker was determined. Survival and growth were the end points measured.

3.3 FORTY-EIGHT-HOUR ACUTE TEST OF THE TOXICITY OF PORE WATERS USING *Ceriodaphnia dubia*

Bioassays on pore water using *Ceriodaphnia dubia* were based on IT Testing SOP B3.0, which is included in Appendix B. IT's procedure is modeled on that of EPA (Peltier and Weber, 1985). Samples were collected in two events (Table A-1), but only one set of assays were run (Table A-3).

Ceriodaphnia dubia neonates used in testing were less than 24 hours old at the time of test initiation. Test chambers were 250-ml glass beakers filled to a volume of 100 ml. Four replicates for each pore water sample were prepared, plus laboratory controls of pore water extracted from sediment gathered from Spruce Run Creek. Ten organisms were randomly chosen from the mass cultures with a wide pore pipet and transferred to the test beakers, which were randomly placed in the test area. Test solutions were not renewed during the 48-hour exposure period. The test end point measured was survival at 24 and 48 hours. The organisms were not fed during the test. The test was terminated after 48 hours.

3.4 TEN-DAY ACUTE TOXICITY TEST OF SEDIMENTS USING *Chironomus tentans*

The ten-day bioassays on sediment using *Chironomus tentans* were based on IT Testing SOP D2.0, which is included in Appendix B. IT's procedure is modeled on that of Nebecker et al. (1984) and EPA (Weber et al., 1989). Samples were collected in two events (Table A-1), but only one set of assays were run (Table A-3).

Second instar larvae of *Chironomus tentans* were used in the solid-phase bioassays. Test

chambers were 1-L glass beakers. Each sieved sediment sample was mixed, and 200-ml aliquots were placed in four replicate 1-L glass beakers. Eight hundred ml of RVR was added into each beaker bringing the total volume in each beaker to approximately 1,000 ml. In all test beakers, the water was introduced slowly by pouring gently down the side of the test beakers. The test beakers were randomly placed in the test area. The sediments were allowed to settle overnight. A tongue depressor was attached with a rubberband to the exterior of each beaker, rising vertically past the top of the beaker. To the tongue depressor was attached an air line and Pasteur pipet, which extended 2 to 4 cm below the water surface. Approximately 30 minutes before introduction of the organisms to the test chambers, gentle aeration was begun.

Organisms from the holding tank were randomly chosen and transferred gently with forceps to 2-ounce plastic weighing boats, 20 organisms per boat. The organisms were then transferred to the test beakers by submerging the boats in the water and allowing the chironomids to slide out of the boats. Any organisms observed floating on the surface after introduction to the test chambers were gently drawn into a wide bore pipet and reintroduced under the water surface. After ten days of exposure, the test was terminated. The sediments were passed through ASTM Standard No. 18 sieves, which retained all surviving organisms and were then counted to complete the test.

4.0 STATISTICAL METHODS

All bioassay tests were conducted on 100 percent surface water, sediment, or pore water samples. No dilution series of any samples were used in testing. The results of the exposures of organisms to test waters or sediments were compared to the results of exposure to the laboratory control and the reference samples, SW-OOM, SWO1M, SD-OOM, and SD-O1R. Statistically significant differences were identified as those with $\alpha = 0.95$.

The three brood chronic tests on surface waters using *Ceriodaphnia dubia* yielded proportions. These were compared using Fisher's Exact Test. The seven-day chronic test on surface waters using *Pimephelas promelas* yielded continuous data that were analyzed

using Dunnett's Procedure. Growth data from the chronic tests on surface waters using *Pimephelas promelas* and reproduction data from the chronic test of surface waters using *Ceriodaphnia dubia* were also analyzed using Dunnett's Procedure. Dunnett's Procedure permits multiple comparisons of mean values. Survival data from the pore water test using *Ceriodaphnia dubia* were analyzed using Student's T-Test, which compares the control and reference means to those of the site samples. Survival data from the solid phase tests using *Chironomus tentans* were analyzed using Dunnett's Procedure.

The test for normality was the χ^2 goodness-of-fit test. Data were compared to an expected frequency of occurrence to determine if the data were normally distributed. Homogeneity of variance across samples for each chronic test was determined using Hartley's and Bartlett's Tests. Both tests are similar; however, Bartlett's Test is not as sensitive to the unequal sample sizes that are sometimes encountered in reproduction tests using ceriodaphnids.

APPENDIX B

STANDARD OPERATING PROCEDURES FOR BIOASSAY TESTS

A10.0 PIMEPHALES PROMELAS 7-DAY CHRONIC TEST

A10.1 SOURCE OF ORGANISMS

A10.1.1 Fathead Minnows (Pimephales promelas) to be used in chronic bioassay testing will be cultured at the IT-Edison laboratory.

A10.1.2 Fathead larvae used for testing will be less than 24 hours old at the start of the chronic bioassay testing. Organisms up to 48 hours old may be used, but all must be within 24 hours of age.

A10.1.3 Reference toxicity tests will be performed monthly on in-house cultures and on every lot of organisms obtained from suppliers and used for chronic bioassay testing.

A10.2 HOLDING AND HANDLING OF TEST ORGANISMS

A10.2.1 Culture tanks for fathead minnows shall contain a recirculating filter system which includes a 10 micrometer filter containing carbon.

A10.2.2 Laboratory grade fresh water is the culture water. It consists of Round Valley Reservoir Water passed through a 10 micrometer filter, sterilized under U.V. light, and then passed through a carbon filtration system.

A10.2.3 Dissolved oxygen levels are maintained above 60 percent saturation using oil-free air lines and air stones in all culture and holding tanks.

A10.2.4 Adult breeder fatheads are fed trout chow, tetramin and brine shrimp (Artemia nauplii) two times per day during culturing. Newly hatched fry are fed 150 nauplii per fathead per holding tank over three daily feedings.

A10.3 QUARANTINE

A10.3.1 All eggs and newly hatched fry shall be quarantined for a minimum of 3 days in test dilution water (laboratory grade fresh water).

A10.3.2 Holding waters shall be renewed every other day during quarantine.

A10.3.3 During the quarantine period observations shall be made on behavior and viability. If behavioral abnormalities or excessive mortalities are observed then the entire group will be discarded and another group of organisms will be used for testing.

A10.3.4 Periodic identification of the stock cultures shall be verified using an appropriate taxonomic key. Records of the verification shall be retained along with a few of the preserved specimens.

A10.4 ACCLIMATION

A10.4.1 Laboratory grade fresh water is used as both holding water for the eggs and as dilution water for the test. Therefore, no acclimation period is required.

A10.5 EFFLUENT SAMPLING AND HOLDING

A10.5.1 Effluent samples shall be collected daily from sampling points as specified in the discharge permits of the appropriate regulatory authority.

A10.5.2 Effluent samples shall be composites of 24 hours of discharge with grabs being taken every 30 minutes.

A10.5.3 Effluent samples shall be held and transported at 4°C.

A10.5.4 Effluent samples shall be used for testing less than 24 hours after collection whenever possible, but under no circumstances shall effluent samples be used more than 72 hours after collection.

A10.6 TEST DILUTION WATER

A10.6.1 The test dilution water shall be laboratory grade fresh water, i.e. Round Valley Reservoir Water passed through a 10 micrometer filter.

A10.7 7-DAY CHRONIC TEST

A10.7.1 Test Design

- All test concentrations and controls shall be run in at least triplicate. Controls shall be laboratory grade fresh water.
- The concentrations used in chronic bioassay shall be determined by historical acute toxicity data or by acute toxicity screening tests performed in preparation to initial chronic testing when no historical data exists.
- Test concentrations shall be in an approximate logarithmic progression utilizing a dilution factor of 0.5.
- Test chambers shall be 600 ml polypropylene tri-pour beakers filled to a volume of 500 ml test solution.

- At least thirty fatheads shall be exposed to each concentration and control at a loading rate of no more than 1 fathead per 40 ml of test solution.

- Test temperatures shall be maintained by placing test chambers in a covered, temperature-controlled water bath at 24-26°C. The water depth surrounding the test chambers shall exceed 2.5 cm. The test water temperature shall be recorded continuously or at a minimum in two places at two separate times.

- Light intensity during the test shall be 50 to 100 foot candles, with a 16 hour light and 8 hour dark cycle and an approximately 30 minute phase-in/out period.

- At the beginning of each 24 hour exposure period temperature, dissolved oxygen, and pH shall be measured in one replicate for control, low, medium and high test concentrations. Dissolved oxygen and pH shall be measured at the end of each 24 hour exposure period in one replicate for controls, low, medium and high test concentrations. Conductivity, alkalinity and hardness shall be measured for each fresh diluent or effluent sample.

- Dissolved oxygen levels shall be maintained above 60 percent saturation. When necessary all chambers shall be gently aerated using an oil free source and providing air at a maximum of 1 cc per minute.

- During the test, the fatheads in each chamber shall be fed Artemia nauplii, which are less than 24 hours old, at the rate of 150 nauplii per fathead over three daily feedings.

- Test solutions shall be renewed daily by preparing fresh solutions, pouring off all but 10 cm of the "old" solution, and replacing it with fresh solution. Before replacement, dead animals and excess food shall be removed with a pipette and the number of live organisms counted and recorded.

A10.7.2 Test Completion

- The fathead chronic bioassay shall reach termination 7 days after test initiation. At that time, test solutions shall be poured off to a one-cm depth and replaced with the test diluent. Final survival counts shall be made at this time and recorded.

- Fatheads shall be rinsed in small netted cups with distilled and deionized water. Using forceps, the fatheads

shall then be placed in tared aluminum weighing boats. These boats shall have been dried in a 105°C drying oven for a minimum of 26 hours and cooled to room temperature in a dessiccator for a minimum of 1 hour before weighing. The fatheads and weighing boats shall be dried in a 105°C drying oven for a minimum of 24 hours and cooled to room temperature for a minimum of 1 hour before weighing. Weights shall be measured to the nearest 10 micrograms.

A10.7.3 Test Endpoints

- Providing survival in laboratory grade fresh water controls is at least 80 percent, survival will be used as a test endpoint. LC50 values shall be determined as well as NOEC and LOEC concentrations for this acute endpoint.

- Providing average control dry weight per organism is not less than 0.25 mg then growth shall be used as an endpoint. NOEC and LOEC concentrations shall be determined for this chronic endpoint.

- Tests will be considered valid chronic tests when survival and growth in laboratory grade fresh water controls meet acceptable criteria.

A10.7.4 Data Analysis

- Survival data will be analyzed by calculation of the LC50. This calculation will be done using a method acceptable to the appropriate regulatory agency. This is usually Probit analysis, Litchfield and Wilcoxin analysis, and Graphical or Non-linear interpolation.

- If applicable, an incipient LC50 curve shall be developed.

- Survival NOEC and LOEC values are determined using Dunnett's procedure on the ARCSINE transformed data.

- Growth NOEC and LOEC values are determined using Dunnett's procedure following analysis for normality and homogenity. If the data does not show normal distribution and has a heterogenous variance then the Steel's many rank test will be used for equal number of replicates and the Wilcoxin rank sum test with Bonferroni adjustment will be used to analyze data with unequal number of replicates. A chronic value (ChV) is determined from the growth LOEC and NOEC.

- Only concentrations below the survival LOEC are used for growth analysis.

A10.8 REFERENCES

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B3.0 48-HOUR *DAPHNIA* STATIC DAILY RENEWAL BIOASSAY

B3.1 SOURCE OF TEST ORGANISMS

B3.1.1 Organisms (*Daphnia pulex* or *magna*) to be used in bioassay testing originated from EPA cultures in Duluth, MN. Daphnids are kept in active culture at IT's Edison, NJ facility.

B3.1.2 First instar daphnids (neonates <24 hours old) will be used for the 48 hour test.

B3.2 HOLDING AND HANDLING OF TEST ORGANISMS

B3.2.1 Holding tanks will maintain a low density culture of daphnids to avoid stressing of the organisms.

B3.2.2 Round Valley Reservoir water from Lebannon, N.J., will be used in the holding chambers.

B3.2.3 To prevent undue stress to the test organisms, they will not be subjected to temperature changes of more than 3°C in water temperature in any 12-hour period, nor less than 40% of saturated DO.

B3.2.4 Daphnids will be fed daily.

B3.2.4 Daphnids are observed daily during the holding period with mortality, abnormal behavior, and feeding noted and recorded.

B3.3 ACCLIMATION

B3.3.1 The Daphnids will be acclimated to the test dilution water and temperature by gradually changing the water from 100% holding water to 100% test dilution water over a 24-hour period.

B3.3.2 The test organims will be held in 100% test dilution water at the prescribed test temperature (± 2°C) for at least 48 hours before the test begins.

B3.3.3 If more than 5% of the Daphnids die during the 48 hour acclimation period, that group of Daphnids will not be used for testing. Another group of organisms will be acclimated to the test dilution water. Should the second group of organisms fail the acclimation process, the regulatory agency governing the bioassay will be contacted to determine an acceptable source of dilution water for the test.

B3.3.4 Daphnids producing ephippia will be discarded and production of ephippia will be considered in determining the suitability of a dilution water source for use in bioassay testing.

B3.4 EFFLUENT SAMPLING AND HOLDING

B3.4.1 Effluent samples will be collected from sampling points as specified in the NPDES discharge permit.

B3.4.2 Each day a sample will be collected and maintained at 4°C using ice during the collection and transportation to the laboratory.

B3.4.3 Effluent samples will be used within 24 hours of collection in the bioassay tests.

B3.5 TEST DILUTION WATER

B3.5.1 The test dilution water will be site water taken upstream of the discharge.

B3.6 RANGE-FIND TEST

B3.6.1 Static 24-hour Test

- Five (5) widely-spaced effluent dilutions
- Ten (10) organisms (Daphnia pulex or magna) per dilution at a loading rate not exceeding 10 daphnids/1000 mls test solution.

B3.6.2 The range-find test is used to determine the range of concentrations to be used in the definitive test if historical toxicity data are not available.

B3.7 48-HOUR DEFINITIVE TEST

B3.7.1 Static 48-Hour Test

- Five (5) concentrations of effluent, plus a control, each run in duplicate.
  - The concentrations used will be determined by the range finding test or historical toxicity data.
  - Test concentrations will be a logarithmic series with two (2) concentrations above and two (2) concentrations below the range finding LC_{50}.
- Twenty (20) Daphnids will be exposed to each concentration at the loading rate not exceeding 10 Daphnids/100 mls of test solution.
- Test will be run at 20 ± 2°C.
- Temperature, dissolved oxygen, pH, alkalinity, hardness and conductivity will be measured and recorded daily.

- D.O. levels will be maintained above 40% saturation, with aeration used only if necessary.

- Test concentrations will be renewed daily with fresh effluent from the appropriate 24-hour composite. Daphnids are transferred by pipet into the new test treatments.

B3.8 TEST RESULTS

B3.8.1 Calculation of EC_{50}

- The calculation of the EC_{50} will bedone using methods acceptable to USEPA and the appropriate state regulatory agency.

- If applicable, an incipient EC_{50} curve will be developed.

B3.9 REFERENCES

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A2.0 CERIODAPHNIA SURVIVAL AND REPRODUCTION BIOASSAY TEST PROCEDURE

A2.1 SOURCE OF TEST ORGANISMS

A2.1.1 Organisms (Ceriodaphnia dubia) to be used in chronic bioassay testing will be cultured at the IT's Edison laboratory and originated from EPA cultures at the Duluth, MN facility.

A2.1.2 First Instar Ceriodaphnids (neonates less than 24 hour old) will be used for the test.

A2.1.3 Reference toxicity testing will be performed periodically on all organisms used for chronic testing. Organisms will be subject to acute reference toxicant testing monthly and chronic reference toxicity testing quarterly.

A2.2 CULTURING, HOLDING AND HANDLING OF TEST ORGANISMS

A2.2.1 Stock cultures will be kept in-house and maintained according to appropriate EPA regulations. Each stock cultures will consist of 20 to 30 mixed age daphnids.

A.2.2.2 Holding tanks will maintain a low density culture of daphnids to avoid stressing of organisms.

A.2.2.3 Laboratory grade fresh water (obtained from Round Valley Reservoir, Lebanon, NJ and filtered through a 60 micrometer mesh) is the culture water and test diluent.

A.2.2.4 To prevent undue stress to the daphnid cultures, they will not be subjected to a temperature change of more than 3°C in water temperature in any 12 hour period, nor will the Dissolved Oxygen be allowed to fall below 60% of saturation at any time.

A.2.2.5 Daphnids will be fed daily with the alga Selenastrum capricornutum, in accordance with established feeding procedures.

A.2.2.6 Daphnids will be observed daily during the holding period with mortality, abnormal behavior and feeding behavior noted and recorded.

A2.3 QUARANTINE

A.2.3.1 Adult breeders obtained from reputable suppliers shall be quarantined for at least 10 to 14 days.

A.2.3.2 Holding waters shall be renewed every other day during quarantine for all breeders purchased from outside.

A2.4 ACCLIMATION

A2.4.1 Daphnid neonates to be used in testing will be hatched in culture water (which is also the test diluent). In case the test diluent is the receiving water, adult daphnids will be acclimated to the receiving waters by gradually changing the water in the holding tank from 100% culture water to 100% test diluent over a time period which will not cause excessive stress. Neonates hatched in the receiving water will then be used for testing.

A2.4.2 Adult daphnids will be held in 100% test dilution water at 25 ±/ 2°C for at least 48 hours before the test begins.

A2.4.3 If more than 5 percent of the adults die during the 48 hour acclimation period, that group of daphnids will not be used for testing. Should the second group of organisms also fail the acclimation process, the regulatory Agency governing the bioassay will be contacted to determine an acceptable source of dilution water for the test.

A2.4.4 Adult daphnids producing ephippia will be discarded and production of ephippia will be considered in determining the suitability of a dilution water source for use in bioassay testing.

A2.5 EFFLUENT SAMPLING AND HOLDING

A2.5.1 Effluent samples shall be collected daily from the designated sampling points as specified in the discharge permits of the appropriate regulatory authority.

A2.5.2 Effluent samples shall be composites of 24 hours of discharge with grabs being taken a minimum of every 60 minutes.

A2.5.3 Effluent samples shall be held and transported at 4°C.

A2.5.4 Wherever possible effluent samples shall be used for testing within 24 hours of sample collection, but under no circumstances shall the holding period of the effluent shall exceed 72 hours.

A2.6 TEST DILUTION WATER

A2.6.1 Unless the client's permit specifies otherwise, the test dilution water will be laboratory grade fresh water (obtained from the Round Valley Reservoir and filtered through a 60 micrometer mesh).

A2.7 7 DAY CHRONIC TEST

A2.7.1 Test Design

- The test will run until 60% of the control females have three broods, usually 7 days, up to a maximum of eight days.
- A minimum of 5 test concentrations plus a control is recommended by NJDEP. A second reference water control is optional when a dilution water other than the culture water is used.
- Test concentrations shall be in an approximately logarithimic progression utilizing a dilution factor of 0.3 or 0.5.
- All test concentrations and controls shall be run with 10 replicates. Controls shall be laboratory grade fresh water.
- The concentrations used in the chronic toxicity bioassay shall be determined by historical acute toxicity data or by acute toxicity screening tests performed in preparation to initial chronic testing when no historical data base exists.
- 10 ceriodaphnids shall be exposed to every concentration at a loading rate not exceeding 1 ceriodaphnid per 15 mls of test solution.
- 30 ml polypropylene Tri-pour beakers shall be used as test chambers filled to an effective volume of 15 ml per replicate.
- Test temperatures shall be maintained in a water bath at a temperature of 25° ±/ 1°C.
- Light intensity during the test shall be 50 to 100 foot candles, with a 16 hour light and 8 hour dark cycle and an approximately 30 minute phase-in/out period.
- Dissolved oxygen levels shall be maintained above 60 percent saturation. Effluent may be gently aerated, if necessary, prior to introduction into test chambers.
- Dissolved oxygen is to be monitored at the beginning and the end of each 24 hour exposure period in one test chamber at each concentration and in the control.
- Alkalinity and Hardness will be measured at the beginning of every 24 hour exposure period in all test concentrations and controls.

- Temperature, pH and conductivity are measured and recorded at the end of each 24 hour exposure period in one test chamber at each concentration and in the control. The pH is measured in the effluent sample each day.

- During the test each test chamber shall be fed with 0.1 mls of algal suspension every day.

- Test concentrations will be renewed daily with fresh effluent from the appropriate 24 hour composite. Ceriodaphnids are transferred by pipet into new test treatments.

- The end point of the test will be survival and effect on reproduction.

A.2.7.2 Test Termination

- Tests should be terminated when 60% or more of the control females have produced their third brood up to a maximum of 8 days.

A2.7.2 Test Endpoints

- The test endpoint is survival and growth. LC_{50} values as well as NOEC and LOEC concentrations shall be determined by appropriate methods.

- The test is considered acceptable if:

- The average survival of control daphnids is equal to or excess 80%.

- Reproduction in the controls must average 15 and more per surviving females.

- At least 60% of the surviving females in the controls have produced their third brood within eight days.

- No ephippia are produced in the controls and

- The number of males int he controls and test concentrations will be minimal.

A2.7.4 Data Analysis

- Survival data will be analyzed by calculation of the LC_1, LC_5, LC_{10}, and LC_{50}. This calculation will be done using a method acceptable to the appropriate regulatory agency. This is usually Probit analysis, Litchfield and Wilcoxon analysis, and Graphical or Non-linear interpolation.

- If applicable, an incipient LC_{50} curve shall be developed.
- Survival NOEC and LOEC values are determined by using Fisher's exact test.
- NOEC and LOEC values for reproductive success are determined using Dunnett's procedure following analysis for normality and homogenity. If the data does not show normal distribution and has a heterogenous variance then the Steel's many rank test will be used for equal number of replicates and the Wilcoxon rank sum test with the Bonferroni adjustment will be used to analyze data with unequal number of replicates. A chronic value (ChV) is determined from the growth NOEC and LOEC.
- Concentrations at which there is no survival are excluded from statistical analysis of NOEC and LOEC, but will be included in the calculation of the LC_{50}.

A.3.8 REFERENCES

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D2.0 10-DAY STATIC ACUTE WHOLE SEDIMENT BIOASSAY WITH *Chironomus tentans*

D2.1 OBJECTIVE

This test is designed to assess the acute toxicity of whole sediments to larvae of the midge, *Chironomus tentans*.

D2.2 HUSBANDRY

D2.2.1 Midge larvae will be cultured at IT Corporation (IT), Edison, New Jersey, according to accepted practices as outlined below. A full record of all activities relating to culture of midge larvae will be kept on file. These procedures are adapted from Nebecker, 1984.

D2.2.2 Midge larvae will be raised in static 10-gallon all-glass aquaria. All aquaria will be artificially aerated through a built-in air blower system which provides air for all culture and test systems. The culture tanks will be maintained at 22 degrees C, and will not be allowed to vary more than two degrees in any 12-hour period. Weekly renewals of culture water will be made with moderately hard reconsituted water. Crushed paper towels will be added as needed for substrate. Animals will be fed *ad libitum* on a diet consisting of pulverized cereal leaves and Tetra® flakes.

D2.2.3 Water quality parameters of hardness, alkalinity, dissolved oxygen, pH, conductivity, and temperature will be made on a weekly basis.

D2.2.4 When larvae are required for a test, adult midges will be removed from culture aquaria via an aspirator and placed in 5-liter all-glass breeder aquaria filled to 1 cm with reconstituted water. Eggs will be transferred to aerated

aquaria containing reconstituted water, cereal flakes, and cultured algae.

D2.3 SAMPLE COLLECTION AND HOLDING

D2.3.1 Sediment samples for testing shall be obtained no longer than 30 days prior to the start of the test, and will be stored at 4 degrees C until needed.

D2.3.2 Enough sediment for the entire test shall be obtained at the same time. To remove debris and predators, samples should be passed through a screen with openings no larger than 1 mm in size (Nebecker, 1984; Cairns, 1984). If possible, this procedure should be completed on site, using site water to aid in sifting. Sifted samples will be stored in clean polyethylene containers. Custody of samples shall follow IT standard procedures.

D2.4 CONTROL SEDIMENT

D2.4.1 A suitable control sediment will be used for each test. Control sediment will be collected from clean watersheds according to the same methods as test samples. Each batch of sediment will be tested for larval survivability prior to use in toxicity tests. Any batch which does not encourage greater than 90 percent survival of second instar larvae for ten days upon retest shall be discarded.

D2.4.2 In the event that regulatory agencies require, or it is deemed necessary by IT for the validity of a test, clean reference sediments shall be obtained from the general location of the test sampling.

D2.5 TEN-DAY ACUTE BIOASSAY

Ten day acute static sediment bioassay shall be conducted according to

methodologies recommended by Nebecker, 1984.

D2.5.1 A minimum of three replicate chambers of each sample will be compared to three replicate chambers of control sediment.

D2.5.2 Test chambers shall consist of acid washed one-liter borosilicate glass beakers. Each chamber will be aerated with a glass tipped pipette held above the sediment and at a rate slow enough to ensure the sediment is not unduly disturbed.

D2.5.3 Sample slurry will be added to each chamber to approximately 300 ml. If this does not provide at least 200 ml of substrate, more slurry will be added. The remainder of the beaker will be filled with moderately hard reconstituted water. Each chamber will be allowed to settle for 24 hours prior to introduction of test organisms.

D2.5.4 At the time of set-up, a subsample of sediment slurry will be taken for testing. Percent organic matter and particle size analysis can be conducted on each sample, if requested.

D2.5.5 At the start of the test, 20 second instar midge larvae (Chironomus tentans) will be randomly selected from culture aquaria using a glass pipette. Only larvae of equal size and exhibiting normal vigor will be used in toxicity tests.

D2.5.6 Initial and final test conditions will be measured using standard IT laboratory procedures and will include dissolved oxygen, pH, conductivity, alkalinity, hardness, and temperature. Dissolved oxygen and temperature will be monitored daily.

D2.5.7 The test will be conducted at 20 +/- 2 degrees C. Photoperiod will be 16 hours light / 8 hours dark.

D2.5.8 Organisms will not be fed during the test unless the percent

organic matter of sediment is less than 5 percent ash-free dry weight (Nebecker, 1986). If feeding is required, 0.2 grams of Tetra® flakes will be added to each test chamber at the start of the second day and again on day 7. Feeding will be reduced or discontinued if fungus is observed.

D2.5.9 Water lost to evaporation will be replenished as needed with moderately hard reconstituted water. This water will be added as slowly as is required to ensure the sediment is not disturbed.

D2.5.10 At the completion of the test, the chambers wil be drained to 300 ml and this water discarded. The sediment will be sifted through a 0.5-1 mm mesh screen and counts made of surviving larvae. Larval length and wet weight can be recorded, if requested.

D2.6 DATA INTERPRETATION

Data will be checked for normality and if assumptions are met, will be analyzed with Tukey's test for two samples. Should normality not be obtainable, the non-parametric Mann-Whitney U Test for comparing two samples will be utilized (Sokal and Rohlf, 1981). Significant difference from controls at an alpha $\leq$ 0.5 will be considered to indicate toxicity.

D2.7 QUALITY ASSURANCE/QUALITY CONTROL

All phases of testing shall be audited according to IT standard QA/QC procedures.